THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY

IMPERIAL MYCOLOGICAL INSTITUTE
3 OCT. 1933

Vol. 20, N.S., Nos. 29–36. APRIL, 1933.

29.—EFFECT OF THE CONDITIONS OF STORAGE ON THE VITAMIN D POTENCY AND ON OTHER FEATURES OF CODLIVER OIL. By E. J. Sheehy, F.R.C.Sc.I., B.Sc., Lecturer in Animal Nutrition, University College, Dublin.

30.—A SUGGESTED MODE OF RADIOTHERAPY WHEN LONG-CONTINUED FEEBLE GAMMA RADIATION MAY BE DESIRABLE. By J. Joly, Sc.D., F.R.S.

31.—ON THE COLLOIDAL CALCIUM PHOSPHATE OF MILK. By G. T. Pyne and J. J. Ryan, Department of Dairy Chemistry, University College, Cork. [Communicated by Professor F. E. Hackett.]

32.—AN INVESTIGATION OF THE BEHAVIOUR OF NEON DISCHARGE TUBES IN A FLASHING CAPACITY CIRCUIT BY MEANS OF A CATHODE RAY OSCILLOGRAPH. By J. H. J. Poole, Sc.D. (Plate 44.)

33.—BAST-SAP. By Henry H. Dixon, Sc.D., F.R.S., University Professor of Botany in Trinity College, Dublin. (Plate 45.)

34.—A STUDY OF *PHOMA LINGAM* (TODE) DESM., AND OF THE "DRY ROT" IT CAUSES, PARTICULARLY IN SWEDE TURNIPS. By William Hughes, B.Agr.Sc., Albert Agricultural College, Glasnevin, Dublin. [Communicated by Dr. P. A. Murphy.] (Plates 46 and 47.)

35.—A CLASSIFICATION OF THE BIOLOGICAL ELEMENTS, WITH A NOTE ON THE BIOCHEMISTRY OF BERYLLIUM. By William Robert Fearon, M.B., Sc.D., F.T.C.D., F.I.C.

36.—EXPERIMENTS ON THE SUITABILITY OF SOME RECTIFIER PHOTO CELLS FOR THE MEASUREMENT OF DAYLIGHT. By H. H. Poole, Sc.D., and W. R. G. Atkins, Sc.D., F.R.S., F.Inst.P.

[*Authors alone are responsible for all opinions expressed in their Communications.*]

DUBLIN:

PUBLISHED BY THE ROYAL DUBLIN SOCIETY,
BALLSBRIDGE, DUBLIN.

HODGES, FIGGIS, AND CO.,
20 NASSAU STREET, DUBLIN.

WILLIAMS AND NORGATE,
28 to 30 LITTLE RUSSELL STREET, STRAND, LONDON, W.C. 2.

1933.

Price Seven Shillings.

Royal Dublin Society.

FOUNDED, A.D. 1731. INCORPORATED, A.D. 1750.

The Scientific Meetings of the Society are usually held at 4.15 p.m. on the fourth Tuesday of every month of the Session (November to June).

The attention of authors is directed to the following extracts from the regulations governing the presentation of communications at Scientific Meetings of the Society, and the publication of papers in the Scientific or Economic Proceedings:—

> "An author who desires to present a communication at a Scientific Meeting of the Society shall furnish to the Registrar a fortnight previous to that meeting the title and an abstract (not exceeding 200 words) of his communication, together with an estimate of the time required for presentation.
>
> "An author who desires to have a communication published forthwith as a paper in the Proceedings of the Society shall furnish to the Registrar the full text and necessary illustrations at least a week before the meeting of the Committee of Science at which he desires publication to be considered. Meetings of the Committee of Science are usually held on the second Tuesday of each month from November to June inclusive.
>
> "A paper may be published either in the Scientific Proceedings or the Economic Proceedings of the Royal Dublin Society, as directed by the Committee of Science. The copyright of papers so published shall be the property of the Society.
>
> "Fifty copies of each paper shall be supplied free of cost to the Author or Authors.
>
> "Additional copies may be obtained at Cost Price if applied for by the Author when returning the proofs."

Authors are requested to apply to the Registrar for further information concerning the preparation of papers and illustrations for press.

Authors of biological papers involving extensive numerical observations, are requested to consult the recommendations of the British Association Committee on Biological Measurements, 1927, obtainable from the British Association, Burlington House, W. 1, price 6d. Arrangements have been made whereby original data, too extensive for complete publication, may be deposited at the British Museum (Natural History), or with the Royal Society of Edinburgh, and so be made available for future workers. Authors desirous of depositing data, whether zoological or botanical, in this manner, should communicate with either the Keeper of Zoology, Natural History Museum, S.W. 7, or the General Secretary, The Royal Society of Edinburgh, 22 George Street, Edinburgh, enclosing full bibliographic reference to the publication in which the results are summarised.

No. 29.

EFFECT OF THE CONDITIONS OF STORAGE ON THE VITAMIN D POTENCY AND ON OTHER FEATURES OF CODLIVER OIL.

By E. J. SHEEHY, F.R.C.Sc.I., B.Sc.,
Lecturer in Animal Nutrition, University College, Dublin.

[Read June 26. Published separately December 30, 1932.]

Codliver Oil varies considerably in its vitamin D potency. It is known that the cause of the variation may be related to the source of the oil and the method by which it is prepared (1). Frequently codliver oil is stored for a considerable period before consumption, and in view of the very considerable difference in the nutritive value of the various samples of veterinary oil on the market the effect of the conditions of storage on the vitamin D potency suggested itself as a fruitful line of inquiry.

The special nutritive value of codliver oil depends upon its content of vitamins A and D. Vitamin A is much less stable than D, and is more likely to be adversely affected by methods of preparation and storage which involve heat and oxidation. Hence there is no constant relationship between the vitamin A and D contents of commercial samples of codliver oil (2). When codliver oil is mixed in a mash there is evidence that after four months, at a temperature as low as 32° C., part or the whole of its vitamin A has disappeared. That oxidation changes are responsible for this effect was shown by Hopkins (3), and Drummond, Zilva, and Golding advised the storage of codliver oil in air-tight vessels to prevent oxidation taking place (4). Drastic treatment like the bubbling of air through heated codliver oil definitely reduces its vitamin D potency (5); and Norris, Heuser, and Wilgus (6), by a series of critical experiments, showed that when codliver oil is mixed with meal and stored in that fashion it also loses some of its vitamin D potency. This is detectable only when the oil is added to the meal in minimum protection doses, because when Payne (7) mixed a potent oil at the rate of 1 per cent. in a meal no loss of vitamin D was noticeable over a period of 12 months.

It is not usual, however, to store codliver oil mixed with food for any considerable time, but a very long period may elapse between the date of the extraction of the oil and that of consumption. During this interval, which may extend to years, the oil is stored in wooden or metal containers either full and sealed or partly filled, so that the air is in direct contact with the surface of the oil. To investigate the effect of storage of the oil under different conditions a sample was chosen, fed to chickens to test its potency, and stored for a period. Then

the portions stored in the different fashions were again tested. The original test was made to determine the percentage of oil which it was necessary to add to an all-mash diet in order to protect chicks from rickets.

Experiment I.—Potency of the Original Sample of Codliver Oil.

Two hundred day-old chicks were divided into five lots of 40 each, all of which were fed on the following basic ration throughout the experiment:—

Bran	...	30	parts (by weight).
Pollard	...	30	„ „
Yellow Indian meal ...	...	30	„ „
Separated milk powder ...	...	5	„ „
Extracted soya bean meal ...	...	5	„ „
Ground limestone	...	1	part „
Common salt	...	½	„ „

This meal mixture was available to the chicks throughout the entire day. Each group of chickens was housed in a compartment lighted only through window glass, and codliver oil was added in the following proportions:—

Group I.—Basic ration alone.
Group II.—Basic ration to which was added 0·25 per cent. codliver oil.
Group II (*a*).—Basic ration to which was added 0·25 per cent. codliver oil.
Group III.—Basic ration to which was added 0·5 per cent. codliver oil.
Group IV.—Basic ration to which was added 1·0 per cent. codliver oil.

Group II (*a*) differed from II in that at a particular stage—namely, when the chicks reached the age of five weeks—the hover lamp was put out, and the chicks were forced in moderately severe weather to live without artificial heat.

No difference in the condition of the birds in the various groups was observable up to the age of five weeks, when the chicks in group I began to show signs of rickets, and commenced to lose their previous thrifty appearance. The symptoms developed, and by the seventh week rickets was pronounced in practically all birds of the group. Groups II, III, and IV made similar progress throughout, and did not exhibit rickets at any stage. The average weights of the birds in these three groups at the age of 8 weeks were 1·76, 1·80, and 1·75 pounds, respectively. When, in the fifth week, the artificial heat was withdrawn from group II (*a*) rickets made their appearance, showing that the proportion of codliver oil supplied to that group was very close to the minimum quantity, and was really insufficient to protect from rickets when the conditions of life became unfavourable.

This test clearly showed that the basic ration was rickets-producing, and that a proportion of 0·25 per cent. of the codliver oil selected was capable, under the favourable conditions of the experiment, of providing chicks with sufficient vitamin D.

Storage of the Oil.

The remainder of the sample of codliver oil was, at the beginning of Experiment I, divided into four different portions, and stored as follows:—

Portion I, in a completely filled and sealed transparent white bottle in a dark cupboard.

Portion II, in a completely filled and sealed transparent white bottle exposed to bright light.

Portion III, in a completely filled and sealed tin-lined metal container.

Portion IV, in a wooden barrel partly filled and bored, so that atmospheric air had free access to the air space over the oil.

Portion of the oil was withdrawn from each container at the end of 12 months for Experiment 2, while the remainder was used up at the end of 16 months for Experiment 3.

Experiment 2.—*Comparative efficiency of the four samples of stored oil when used at the rate of* 0·5 *per cent. in the ration.*

Day-old Wyandotte chicks were segregated into four groups of 35 each, and housed in compartments lighted only through window glass. The basic portion of the all-mash diet was the rickets-producing mixture, the formula for which is given under Experiment 1. To this mixture codliver oil was added to the extent of 0·5 per cent., the stored portions I, II, III, and IV being fed to groups I, II, III, and IV, respectively. The chicks in all groups made normal progress, and showed no signs whatsoever of rickets at the age of eight weeks, when the average weight per individual of each group was as follows:—

Group I. (oil stored in sealed bottle in dark), 1·67 lb.
Group II (oil stored in sealed bottle in light), 1·61 lb.
Group III (oil stored in sealed metal container), 1·59 lb.
Group IV (oil stored in barrel partly filled), 1·63 lb.

According to this test the four portions of oil stored in the different ways showed, after 12 months, the same vitamin D potency when fed at the rate of 0·5 per cent. of the basic ration. Possibly, however, the oil was fed in quantity which exceeded the minimum requirement in each case, so that a possible difference in potency may not have become apparent. It became necessary, therefore, to perform another experiment with a reduced proportion of codliver oil in the mash.

Experiment 3.—*Comparative efficiency of the four samples of stored oil when used at the rate of* 0·25 *per cent. in the ration.*

This was conducted on exactly similar lines to Experiment 2, with the single exception that the codliver oil was used in the proportion of 0·25 per cent. instead of 0·5 per cent., as in Experiment 2. The test was begun 4 months later than the preceding one—*i.e.*, 16 months after the date of storing the oil.

No trace of rickets appeared in any group, and at the age of eight weeks the average weights of the comparable groups were as follows:—

Group I (oil stored in sealed bottle in dark), 1·60 lb.
Group II (oil stored in sealed bottle in light), 1·64 lb.
Group III (oil stored in sealed metal container), 1·63 lb.
Group IV (oil stored in barrel partly filled), 1·59 lb.

Two fowl were selected from each group at this stage and the right femur of each was analysed for lime, with the following result:—

	Per cent. of CaO in dry fat-free bone.
Group I—Bird 1	26·7
Bird 2	25·6
Group II—Bird 1	26·2
Bird 2	25·7
Group III—Bird 1	25·7
Bird 2	25·8
Group IV—Bird 1	26·6
IV Bird 2	25·6

It is clear from these results that when the codliver oil was fed to an extent as low as 0·25 per cent. the four portions of oil stored in different ways showed an equal vitamin D potency. The test conducted on the original codliver oil—Experiment 1—showed that the proportion of oil used in this experiment—namely, 0·25 per cent.—was extremely close on the minimum quantity which sufficed to prevent rickets in chicks. It may be concluded, therefore, that from the point of view of practical application at any rate the codliver oil lost none of its vitamin D potency during 16 months of storage, and that no difference in vitamin D potency resulted from storing in the different ways described.

Other Tests Applied.

The oil previous to storage, and again after a 16 months' interval, was examined for colour, unsaponifiable matter, and free fatty acid. For comparative purposes the original colour was matched with a particular dye, which was then used to determine a possible change in the colour of the oil after storage. The table hereunder gives the results of the various determinations:—

	Original oil.	Portion I. Sealed bottle in dark.	Portion II. Sealed bottle in light.	Portion III. Sealed metal container.	Portion IV. Barrel partly filled.
Density of colour . . .	1	1	0·5	1	2
Per cent. unsaponifiable matter	·78	·80	·77	·81	·76
Per cent. free fatty acid (expressed as oleic acid).	·506	·834	·848	·822	1·280

The original colour is expressed in the table by the figure 1. Portions I and III, stored in the dark away from air, maintained the colour unchanged. Portion II became bleached, its density of colour being expressed as half that of the original. On the other hand, portion IV became more intensified in colour, the expression of its density having been put at twice that of the original oil. There was no significant change in the unsaponifiable matter. All portions of oil had, however, increased in fatty acid content during the interval of storage. The increase was similar in portions I, II, and III, being about 60 per cent., while in portion IV the fatty acid content was raised by approximately 150 per cent.

Stored commercial veterinary codliver oil of good quality does not alter its colour materially, if at all, provided light and air are excluded. The effect of light is to bleach the oil, and to reduce its vitamin A potency (8). It is, therefore, important to store the oil in opaque containers. The contact of the oil with the wood of the barrel may possibly be responsible in whole or in part for the increase in colour and acidity in portion IV, and experiments are in progress to test this point.

I have pleasure in gratefully acknowledging the assistance given by Miss K. Shiel in connection with one of the experimental tests on chicks, and by Mr. B. J. Senior in the laboratory determinations.

Summary and Conclusions.

1. A good quality sample of codliver oil does not materially alter in its vitamin D (anti-rachitic) potency when stored for a period of 16 months—(1) in a completely filled and sealed transparent bottle exposed to light, (2) a similar bottle kept in a dark cupboard, (3) a completely filled and sealed tin-lined metal container, and (4) a barrel partly filled with oil.

2. Contact of the oil with the tin or wooden lining of a container, or with the air over the oil in a partly filled vessel, does not appreciably reduce the vitamin D content of codliver oil.

3. Since light bleaches codliver oil and reduces its vitamin A potency, and contact with air possibly increases the rate of formation in it of free acid, it is important that the oil be stored in opaque vessels, and that the containers be completely filled, so as to exclude air.

REFERENCES.

(1) DRUMMOND and HILDITCH.—"The relative value of codliver oil from various sources." Empire Marketing Board, **35** (1930).

(2) CLARE and SOAMES.—"Relative content of the fat soluble A and D in a series of codliver oils." *Lancet*, **214**, 150–182 (1928).

(3) HOPKINS.—"The effects of heat and aeration on the fat soluble vitamin." Biochem. Jr., **14**, 725 (1920).

(4) Drummond, Zilva, and Golding.—"Use of codliver oil in the feeding of farm animals." Jr. Agr. Sc., **13**, 153 (1923).

(5) Heuser and Norris.—"The Effect of heat and exposure to air upon the stability of Vitamin D." Amer. Jour. Dis. Children, **38**, 481 (1929).

(6) Norris, Heuser, and Wilgus.—"Effect of storage in finely divided feeds on the stability of the D vitamin of codliver oil." Memoir 126, Cornell Univ. Agr. Exp. Stn. (1929).

(7) Payne.—"The Anti-rachitic potency of codliver oil when mixed and stored in feed six and twelve months." 4th World's Poultry Congress, Section B, p. 294 (1930).

(8) Holmes and Piggott.—"Effect of light on the vitamin A content of codliver oil." Boston Med. and Surg. Jour., **195**, 263.

No. 30.

A SUGGESTED MODE OF RADIOTHERAPY WHEN LONG-CONTINUED FEEBLE GAMMA RADIATION MAY BE DESIRABLE.

By J. JOLY, Sc.D., F.R.S.

[Read June 26. Published separately December 13, 1932.]

The existence of a penetrating radiation of extra-terrestrial origin and detectable, so far as observation goes, all over the surface of the earth, and at all altitudes, is now well-established.

It has been suggested that variations in the strength of such rays from time to time may affect the wellbeing of organic life; and that the apparent increase of malignant disease is, conceivably, connected with a general decrease of such rays within recent times.[1]

It is perhaps not without bearing upon this speculative view that in certain mines—notably in certain lead mines—a comparative immunity from malignant disease has been observed. The intensity of gamma radiation in such mines deserves quantitative examination.

The object of the present note is to suggest a method whereby continuous and uniform gamma radiation of any desired strength may be readily obtained for surgical purposes.

The direct application of a radioactive ore—*e.g.*, uraninite—is suggested, the strength of the radiation being determined by the amount of ore brought into use.

To render this system easily carried out the ore is, in the first place, brought into a very fine powder. The powdered ore is then made into a paint, and while in the semi-fluid state it is brushed on to any light fabric whereto it adheres securely.

The fluid used in this paint is prepared as follows:—10 g. of cellulose acetate is dissolved in 100 c.c. of acetone; this is not inflammable. Into this fluid the finely pulverised uraninite is stirred, yielding a black paint-like fluid. This is applied with a paint-brush, so that the fabric is uniformly blackened. The fabric so treated is left face upwards exposed to the air while the acetone is evaporating. This procedure, which was suggested by Professor H. H. Dixon, F.R.S., has proved, physically, entirely satisfactory, the finely divided uraninite adhering firmly to the fabric.

The gamma radiation emitted by such a radioactive fabric may be estimated by bringing it near to a charged gold-leaf electroscope.

It is desirable to leave a wide margin of the fabric untouched—say, 3 or 4 cm.—to enable the radioactive cloths to be readily handled.

If it is thought necessary to increase the intensity of the dosage, it is only requisite to superimpose a second radiating fabric. Or it might be desirable to prepare, in the first instance, radioactive cloths of varying strength. This is easily accomplished by putting more than one application of the radioactive "paint' on to the cloth.

---

[1] See note in "Nature," July 29th, 1929, by the present writer.

No. 31.

ON THE COLLOIDAL CALCIUM PHOSPHATE OF MILK.

By G. T. PYNE and J. J. RYAN,
Department of Dairy Chemistry, University College, Cork.

[Communicated by Professor F. E. Hackett.]

[Read November 22. Published separately December 30, 1932.]

The so-called acidity of milk, determined by titration with alkali to a faint pink with phenolphthalein, and usually expressed for pedagogical purposes as a percentage of lactic acid, is known to be a much more complex value, and cannot be strictly said to be more than a measure of the quantity of alkali required to bring the complex mixture of buffers — phosphates, citrates, proteins, &c. — which largely constitute milk, from an original pH of about 6·6 to 8·3. The phenolphthalein end point is somewhat indefinite, owing to fading, and may be considerably displaced on dilution of the sample, phenomena attributed by Van Slyke and Bosworth (7) to the disturbing effects of soluble calcium salts. According to these writers a slow reaction of the type

$$3CaHPO_4 \rightarrow Ca_3P_2O_8 + H_3PO_4$$

occurs during titration, the originally formed dicalcic salt decomposing to give the more insoluble tricalcic phosphate and free phosphoric acid, which in turn undergoes a similar change on continued neutralisation in the presence of sufficient calcium salts. They found that the addition of a soluble oxalate (2 per cent. of a saturated solution of the neutral potassium salt was recommended) by removing calcium did not permit the neutralisation to pass beyond the stage of forming K_2HPO_4, and obviated the indefiniteness of the end point and its marked sensitiveness to dilution. The acidities determined in this way were found to be only about one-half those obtained in the absence of oxalate, and were noticed by them to agree closely with the acidities of (subsequently oxalated) milk serum prepared by passage of milk through a Chamberland filter. In harmony with these results it was found that the "insoluble" residue of casein and colloidal calcium phosphate which, together with a small amount of the corresponding magnesium salt, remained on the walls of the filter was practically neutral to phenolphthalein, and thus did not contribute appreciably to the acidity of milk. Examination of larger amounts of this colloid complex prepared by high-speed centrifuging of milk confirmed these properties, and

led Van Slyke and Bosworth to regard it as a mixture of neutral calcium caseinate and colloidal dicalcium phosphate ($CaHPO_4$).

In the course of some investigations in another connection the writers found it necessary to make a number of titrations on oxalated milks, and, as results were sometimes obtained which seemed at variance with those of Van Slyke and Bosworth, it was decided to examine the matter further.

Titrations both of mixed milks and of those of individual cows showed that samples were sometimes met with in which the addition of 2 per cent. of saturated potassium oxalate was apparently not sufficient to remove entirely the disturbing calcium salts, as much lower acidities were obtained on increasing the amount of oxalate to, say, 4 per cent. In a few cases the addition of the latter amount of oxalate caused the milks to become definitely alkaline to phenolphthalein. A few typical results with various kinds of milks are shown in Table 1.

TABLE 1.

Acidity as c.c. N/10 alkali per 100 c.c. milk.

Sat. Potass. Oxalate ...	None.	2 %	4 %
Milk A	—	10·4	10·2
Serum „	—	10·6	10·4
Milk B	—	9·5	9·0
Serum „	—	10·1	10·0
Milk C	—	9·1	7·6
Serum „	—	9·5	9·5
Milk D	—	8·0	4·5
Serum „	—	7·0	7·3
Milk E	15·0	4·9	− 1·5
Serum „	—	4·5	4·4
Milk F	14·0	6·3	− 0·5
Serum „	—	3·7	3·7
Milk G	—	3·6	− 1·0
Serum „	—	4·0	4·0
Milk H	—	2·5	− 2·3
Serum „	—	3·7	3·5

It may be seen that the acidities of milk and corresponding rennet[1] serum (both containing 2 per cent. sat. oxalate) agree fairly well in a number of cases as observed by Van Slyke. The agreement appears, however, to be somewhat fortuitous, as further additions of oxalate frequently show the milk calcium to have been apparently incompletely removed at the earlier stage. It might be held that 2 per cent. of oxalate represents roughly the average amount necessary to precipitate completely the soluble calcium of most milks, without appreciably attacking the supposedly neutral colloidal casein calcium phosphate complex, and that the increased alkalinity which results from

[1] The more easily prepared rennet serum was used instead of the Chamberland serum employed by Van Slyke. The difference in salt content is negligible.

further addition of oxalate is due to attack and decomposition of the colloid (which must, therefore, be assumed to consist at least partly of tribasic phosphate). The following typical values (Table 2) obtained for the acidities of milk and rennet whey do not offer much support for this view, as they show that the fall in titratable acidity of milk is gradual and progressive with increasing amounts of oxalate, and that the minimum quantity of oxalate for the complete removal of calcium from whey—less than 1 per cent. of saturated aqueous potassium salt—is much lower than that recommended for use with milk (2 per cent.):—

TABLE 2.

Per cent. Sat. Potass. Oxalate soln. added.	Acidity of Milk c.c. N/10 NaOH per 100 c.c.	Acidity of Whey c.c. N/10 NaOH per 100 c.c.	Contribution of casein-calcium phosphate complex to acidity.
0·00	17·5	10·1	7·4
0·25	16·2	8·7	7·5
0·50	15·2	7·5	7·7
0·75	13·7	7·4	6·3
1·00	12·2	7·4	4·8
1·50	10·5	7·4	3·1
2·00	7·9	7·4	0·5
2·50	5·2	7·4	– 2·2
10·00	5·3	7·4	– 2·1

It seems difficult to avoid the conclusion that the milk acidities shown on addition of 2 per cent. sat. oxalate are devoid of special significance, and it appears probable that the continued fall in acidity on the addition of oxalate beyond about 0·75 per cent. is due to the gradual conversion of colloidal tricalcium phosphate into tribasic alkali phosphate. An idea of the amount of tricalcium phosphate present in milk can be obtained from the values in the final column of Table 2, which show the acidity of the casein calcium phosphate complex to have diminished by nearly 10 c.c. N/10 per 100 c.c. milk on adding sufficient oxalate. Assuming that at the phenolphthalein end point the phosphate is mainly present as K_2HPO_4, and that the titratable acidity of the casein itself is not markedly affected by its interaction with oxalate, this value would correspond to about 0·16 g. $Ca_3P_2O_8$, containing about 0·086 g. CaO. This amount of lime is somewhat greater than that calculated by Porcher and Chevallier (6) to be present in the form of colloidal phosphate in average milk, and, although it may not be strictly correct to compare milks of different origins, these figures at least afford some support for the view that a considerable, if not the greater, part of the colloidal phosphates are tribasic.

The exact nature of the inorganic colloid of milk has been the subject of much investigation, and various views have been held. Van Slyke and

Bosworth (8) considered the colloid to be dicalcium phosphate ($CaHPO_4$), Hoffmann and Gortner (2), and Porcher (5) a mixture of di- and tricalcium phosphates. Grimmer and Schwarz (1) find the mineral matter of separator slime to be largely dicalcium phosphate, though it is probably not correct to take this easily deposited phosphatic fraction as typical of the whole inorganic colloid content of milk. Recent work by Pietri (4) leads him to conclude that the colloid is tricalcium phosphate. It is clear that no very definite consensus of opinion is yet available on the matter. The experiments with oxalate, described earlier in this paper, seemed to indicate in a qualitative way that tricalcium phosphate predominated in some cases, but, as marked differences were often found in the behaviour of various milks with oxalate (cf. Table 1), it seemed possible that the type of phosphate predominating might not be the same in all. To examine the matter further, and to determine if possible how far such variation in colloid composition might occur, the casein-calcium phosphate complex was separated by prolonged high-speed centrifuging from two very different types of milk (as regards their reaction with oxalate) and submitted to analysis.

The milks used were first skimmed by passage through a creamery separator, and the skim milk then allowed to remain for 4 hours in the bowl of the separator at 7,200 r.p.m. The thick slimy deposit of casein and phosphate was removed, well mixed, and submitted to analysis. A sample of the almost whey-clear liquid remaining in the bowl was also analysed, so as to make it possible to allow for its contribution to the moist bowl solids. This procedure of obtaining the composition of the net bowl solids was adopted rather than that of Van Slyke and Bosworth (viz., washing with alcohol) in order to obviate any possible hydrolysis or change of composition. The results of analysis are shown in Table 3. Milk I. ("Normal" sample) had an initial acidity of 16·8 c.c. N/10 per 100 c.c., falling to 4·5 c.c. N/10 on addition of 4 per cent. of saturated potassium oxalate; while for Milk II. ("Abnormal"), the corresponding values were 12·7 c.c. N/10 and −4·2 c.c. N/10, respectively—*i.e.*, this sample became strongly alkaline on addition of oxalate. The pH of the first milk was 6·6 and that of the second 6·8 (determined colorimetrically).

TABLE 3.

	Milk I ("Normal").			Milk II ("Abnormal").		
	Comp. of dried deposit Per cent.	Combined with casein Per cent.	Present *as* phosphate Per cent.	Comp. of dried deposit Per cent.	Combined with casein Per cent.	Present *as* phosphate Per cent.
Casein	90·0	—	—	85·4	—	—
CaO MgO	4·35 ·17	1·53	3·06	5·07 0·26	1·56	3·81
P_2O_5	4·04	1·65 (1·47)	2·39 (2·59)	4·85	1·56 (1·39)	3·29 (3·46)

Of the total calcium and phosphate content of the deposit a certain proportion is due to calcium caseinate. The amounts which must be allowed for this combination have been calculated on the assumption that the lime content of casein is related to its hydrogen ion content in accordance with the findings of Palmer and Richardson (3), *e.g.*, 1·50 per cent. CaO at pH 6·45, and 2·00 per cent. CaO at pH 7·10, &c., and that its phosphorus content is 0·8 per cent. (average value for a number of observers). From the net amounts of calcium and phosphorus available for the formation of calcium phosphate it is clear that the latter is largely the tricalcium salt in both milks ($Ca_3P_2O_8$ gives $CaO/P_2O_5 = 1{\cdot}18$, and $CaHPO_4$ gives $CaO/P_2O_5 = 0{\cdot}79$). Some dicalcium phosphate may also be present, but the analytical results for the phosphates, depending as they do on the amounts of calcium and phosphorus assumed to be combined with casein, are not sufficiently accurate to allow of its exact determination. Thus, the figures for P_2O_5 in brackets in Table 3, calculated on the assumption that casein contains 0·71 per cent. phosphorus—Van Slyke's value—instead of the value 0·8 per cent. taken above, make the presence of some dicalcium phosphate much more definite, and illustrate at the same time the difficulty of arriving at exact values.

The main analytical differences in the composition of the deposits from the two milks appear to lie in the different proportions of ash to casein—the "abnormal" sample being relatively richer in ash, and thus in colloidal phosphate, than the "normal." Considerable variations have been observed in the amounts of colloidal phosphate in milk (cf. Van Slyke and Bosworth (8)), and it would appear that it is largely to this factor, and not to differences in the type of colloidal phosphate present, that the varied behaviour of different milks with oxalate must be attributed. Initial differences in hydrogen ion concentration of the various samples are of course a contributing factor.

SUMMARY.

1. The acidities obtained for fresh milk to which a soluble oxalate has been added are not related in any obvious manner to the initial acidity either of the unoxalated milk or of the milk serum, but appear to depend largely on the colloidal phosphate content of the milk.

2. The colloidal phosphate present appears to be mainly tricalcium phosphate.

REFERENCES.

1. GRIMMER and SCHWARTZ (1925).—Milchwirtschaftl. Forsch, **2**, 163.

2. HOFFMANN and GORTNER (1925).—Colloid Symposium Monograph II. New York. Quoted Porcher (q.v.).

3. Palmer and Richardson (1925).—Colloid Symposium, Monograph II. New York. Quoted Porcher (q.v.).

4. Pietri (1931).—Comp. Rend. Acad. Sci., **193**, 1041.

5. Porcher (1930).—Le Lait au point de vue colloidal, p. 353 (Lyon).

6. Porcher and Chevallier (1923).—Le Lait, **3**, 188.

7. Van Slyke and Bosworth (1914).—New York Agr. Exp. Sta. Rep.—Tech. Bull., No. 37.

8. Van Slyke and Bosworth (1914).—New York Agr. Exp. Sta. Rep.—Tech. Bull., No. 39.

No. 32.

AN INVESTIGATION OF THE BEHAVIOUR OF NEON DISCHARGE TUBES IN A FLASHING CAPACITY CIRCUIT BY MEANS OF A CATHODE RAY OSCILLOGRAPH.

By J. H. J. POOLE, Sc.D.

(Plate 44.)

[Read November 22, 1932. Published separately January 18, 1933.]

In two previous papers (Sc. Proc. R. Dub. Soc. (N.S.), **19**, 17 (1928), and Phys. and Opt. Soc. Discussion (Photo-Electric Cells and their Applications), 1930) a method of using a flashing neon tube circuit and a photo-electric cell as a convenient field photometer has been described. This method seems to have advantages in certain cases, but, as might be expected, some difficulties have been experienced with the amount of regularity which can be obtained from the neon discharge tube. In this paper, accordingly, a more detailed study of the actual behaviour of the tube has been undertaken with the hope of designing a tube of greater regularity in behaviour than the commercial tubes previously used, and, although this object has not yet been attained, certain conclusions as regards the flashing phenomena in the tube have been reached which are, I think, worthy of publication.

To investigate the characteristics of high speed phenomena of this nature the easiest instrument to employ is a cathode ray oscillograph. So far as I am aware the only previous serious attempt to employ this method is that described by Dr. W. A. Leyshon (Proc. Phys. Soc., **47**, p. 157). She employed a Western Electric Cathode ray oscillograph, but as most of her records were obtained by tracing, and the arrangement was such that the records of individual flashes were usually superimposed, her results give us little information as regards the regularity of the flashing.

Furthermore, no attempt was made to obtain the voltage/time characteristic of the tube, which is probably the best way of attacking our problem, as it is the behaviour of the tube during the dark period which nearly completely determines the rate of flashing when this rate is sufficiently low to render the time of actual discharge negligible. As in our case the maximum rate of flashing should not exceed three per second, so that the flashes may be counted visually, this condition is usually satisfied, except in certain cases with comparatively large capacities and special types of tube. I should like to mention here that I do not intend these remarks to be in any sense a criticism of Dr. Leyshon's valuable paper, but only to show the difference between the two problems attacked, and the consequent difference in the methods employed.

As it was desired to use photographic recording it was decided to employ a von Ardenne Oscillograph, the brightness of the spot obtained with this tube rendering it more suitable than the Western Electric model. This tube is fitted as usual with four electrodes, and at first it was intended to use a pair of these to furnish a time base by charging a capacity connected across the plates with a constant current obtained from a diode. Dr. Leyshon used this method in obtaining curves for discharge current against time (*loc. cit.*). The method was, however, not found satisfactory owing to the finite resistance between the oscillograph plates, and accordingly the older plan of using a uniformly revolving plane mirror to reflect the image of the oscillograph spot into the camera was employed. The mirror was driven by a variable speed electric motor through a belt and worm reduction gear, and by this means any desired time scale could be obtained on the photographic plate.

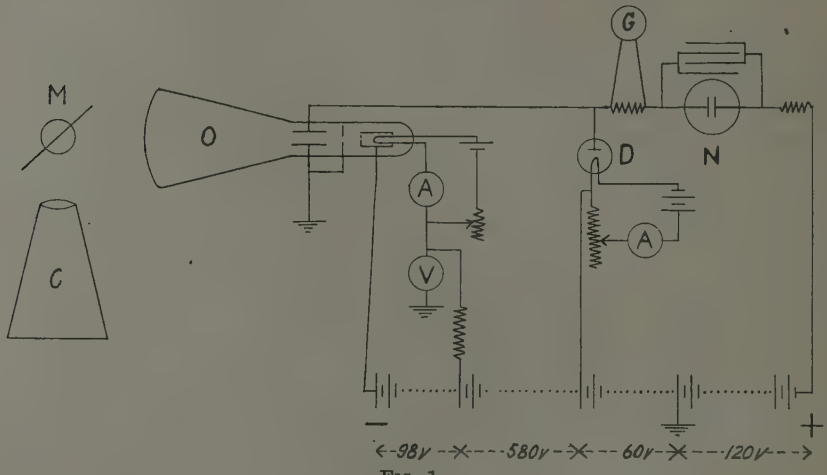


Fig. 1.

Fig. 1 shows the lay-out first adopted. N is the neon tube circuit, D a diode used as a current limiter. The charging current was measured by means of a shunted moving coil galvanometer whose sensitivity could be varied by altering the magnitude of the shunting resistance. One plate of the oscillograph O is connected as shown to the isolated portion of the flashing circuit, the other plate being earthed. M is the revolving plane mirror, and C the camera. This was of quarter plate size, with a lens of f/4·5 aperture. Golden Iso-Zenith plates 1400 H and D were found to be most suitable. For convenience in drawing, the axis of rotation of the mirror and the plane of the oscillograph plates are shown parallel, but actually they are perpendicular, so that, as the mirror axis was vertical, the displacements of the oscillograph spot should be vertical also. The other pair of oscillograph plates usually had a constant potential of about 70 volts across them to correct for the deviation of the cathode beam produced by the vertical component of the Earth's Field.

It would have been mechanically inconvenient to have arranged that the beam should be actually along the total field, although this method would theoretically be preferable.

It is not possible to have the full potential across the oscillograph plates, as this would produce too large a deflexion. Futhermore, as it is the variations in this potential which are important, it is not desirable to reduce this potential by a series capacity method. The most convenient method is to earth some intermediate point of the H.T. battery supplying the diode and neon tube, so that the mean potential of the isolated oscillograph plate is earth potential. The required point can easily be found by trial. By this method we take full advantage of the sensitivity of the oscillograph.

Unfortunately this circuit was found to be unsatisfactory for small currents and small capacities in the flashing circuit. This is due to the fact that, owing to the oscillograph being filled with argon at about ·01 mm. pressure, it does not function as a purely-electrostatic device, a small current flowing between the two deflecting plates when they are at different potentials. The ionisation of the argon is due not to this potential but to the cathode beam. This current is probably not large, but it is evidently sufficient to upset totally the conditions in the flashing circuit when the charging current is of about the same size, and it was found that the neon circuit was entirely erratic when the shunting capacity was smaller than 0·05 microfarad and the rate of flashing not excessively high.

It was, accordingly, necessary to insert a triode valve between the oscillograph and the flashing circuit as shown in Fig. 2. The isolated portion of this circuit is connected to the grid of the valve, and an anode resistance coupling used for the oscillograph. Two conditions have to be satisfied:—

(1) The grid must always be at a sufficiently large negative bias, so that the grid current is zero.

(2) The swing of the flashing voltage must not be large enough to carry the grid potential round the lower bend of the grid volts/anode current characteristic of the valve.

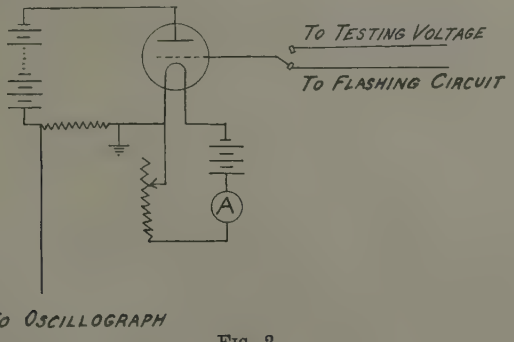


Fig. 2.

For the commercial neon lamp used an Osram P 625 A was found to satisfy both these conditions, as the swing was not usually greater than 34 volts, but for other tubes it was found necessary to use an Osram L S 5 A, as swings up to nearly 70 volts were obtained. As the plate current of both these valves is large, ranging up to 40 milliamperes, it is obvious that the leakage current across the oscillograph plates will have a negligible effect. It is, however, necessary to standardize the oscillograph valve circuit by applying known negative voltages to the grid of the valve and observing the deflections of the oscillograph spot produced. In practice it was found convenient, when measuring the average striking and extinction voltages of a tube, not to resort to photographic registration but to observe with a cathetometer the lowest and highest positions of the spot in the tube, and then by applying known voltages directly to the grid determine what difference in voltage these readings represented. It is necessary to keep the filament current of the valve accurately constant, as the sensitivity plainly depends on this quantity. The requisite negative bias on the grid is obtained by adjusting the earth point on the flashing circuit H.T. battery. A separate insulated H.T. supply must be used for the coupling valve,

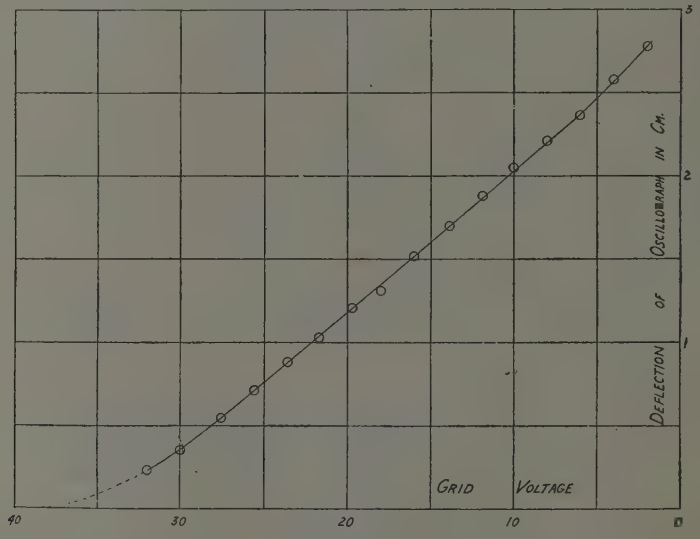


Fig. 3.

as in the circuit shown the average mean potential of this battery alters. Figure 3 shows the voltage calibration curve of the oscillograph using the P 625 A valve, filament current 225 milliamps., anode voltage 120 volts, anode resistance 2000 ohms. It is very nearly linear except for a slight curvature at either end. The error introduced by neglecting this curvature is not large, but care must be taken for the grid negative voltage never to exceed about

35 volts. The position of the centre of the oscillograph spot is plotted, the top and bottom of the spot being measured with the cathetometer and the mean value taken. As usually connected the valve only increased the sensitivity of the oscillograph slightly.

RESULTS.

1.—*Variation of voltage range with current and capacity.*

It was found in agreement with previous investigators (see Appleton, Emeleus, and Barret, Proc. Camb. Phil. Soc., **22**, p. 434, 1924, also W. Clarkson, Phil. Mag., Nov. and Dec., 1927) that the voltage range is a function both of the shunting capacity and of the charging current. Usually the range increases with decreasing capacity and current. Figure 4 illustrates this point. In it the difference between the striking and extinction voltages is plotted against the shunting capacity in microfarads, the scale of the abscissae being logarithmic.

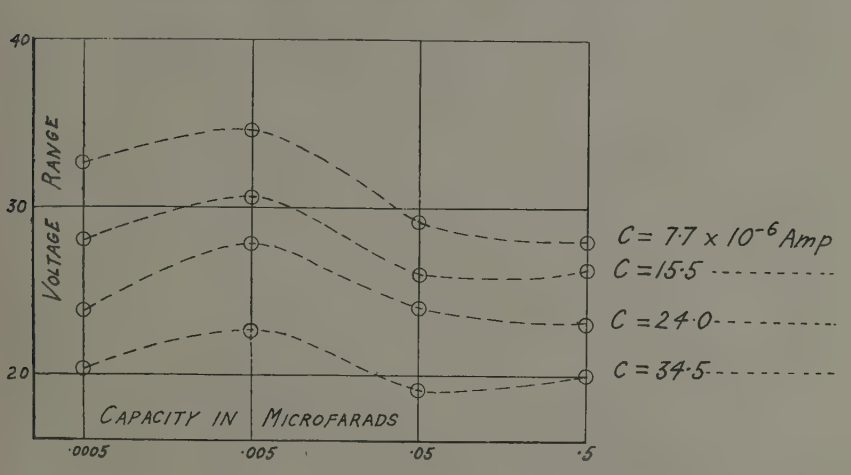


FIG. 4.

Each separate curve gives the appropriate values for the constant charging current shown beside it. It will be noticed that while for a fixed capacity the range invariably increases with decreasing current, there is a tendency for the range at constant current to decrease slightly again for the smallest shunting capacity used. These values were obtained for a commercial Neon letter lamp with bar and plate electrodes, the plate electrode being the cathode. Other tubes gave similar results. The change of voltage range with current would appear to invalidate the fairly accurate proportionality between rate of flashing and charging current previously found, if it were not for the modifying effect

of the discharge currents existing in the tube during the dark periods as discussed in the next paragraph.

2. *Existence of appreciable currents through the neon tube before actual flashing occurs.*

If the charging current were absolutely constant and the resistance of the neon circuit infinite during the dark period, the voltage/time characteristic should be linear. In no case, however, was this found to be true, all the records showing a fall in the slope of the characteristic as the voltage rises.

The possibility that this effect is due to the charging current decreasing as the voltage rises may be, I think, ruled out for several reasons:—(*a*) The valve used as a diode was a bright emitter R 5 triode valve with the grid connected to the plate and the filament run very cold. Under these conditions a small voltage would produce saturation. (*b*) The curvature is least apparent when the capacity is largest, and consequently the charging current greatest. There

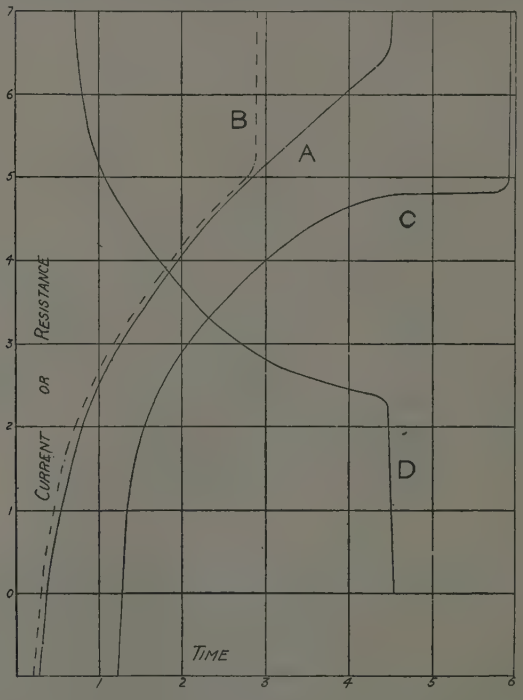


FIG. 5.

Time Scale	Curves A, B, and D.	1 unit = 1 sec.
,, ,,	,, C.	,, ,, ,, 0·2 sec.
Current Scale	,, A, B, and C.	,, ,, ,, 10^{-8} amp.
Resistance Scale	,, D,	,, ,, ,, 10^{9} ohms.

should, however, be more chance of the diode being unsaturated under these conditions. (*c*) Although it was found difficult to keep the reading of the galvanometer absolutely steady during the flash, the variations were certainly not greater than 5 per cent. (*d*) Increasing the voltage drop across the diode had no effect on the curvature of the records.

Prints 1 to 4, Plate 44, show the type of curvature observed. It is possible from the records to obtain the rate of increase of voltage of the shunting capacity with time, and hence determine the actual current flowing into the capacity if its size is known. By subtracting this current from the diode current we get the value of the leakage current in the neon lamp circuit during the dark period. This operation has been carried out for prints 1, 3, and 4, and the results are shown in Fig. 5. These three records were obtained using a commercial neon lamp with the cap and steadying resistance removed in the usual way. The lamp was buried in paraffin wax, as previously described, to avoid surface leaks and charges on the outside of the glass. Curves A and B were obtained with a shunting capacity of ·005 microfarad and a diode current of $6{\cdot}8 \times 10^{-8}$ amp., curve C with a capacity of ·0005 microfarad and a current of $5{\cdot}0 \times 10^{-8}$ amp. To show that the effect is not due to a leak between the two electrodes of the lamp, curve D has been plotted showing how the effective resistance of the circuit in curve A falls as the voltage rises. The flashing range for curve A is about 120 to 152 volts. It will be seen that at the commencement of the charging period there is apparently a reverse current in the lamp. Too much weight cannot be given to this point, as it may be a spurious effect due either to the real capacity of the shunting condenser at the start being reduced owing to hysteresis in the dielectric, or possibly to small grid-currents in the coupling valve increasing the diode current momentarily. Further investigation on these points is desirable, using some more direct method of measuring the dark current, but the existence of these currents is, I think, certain, agreeing as the results do with the previous work by Clarkson (*loc. cit.*).

3.—*Irregularity of flashing phenomena with small shunting capacities.*

Prints 1 and 7 both show how erratic the flashing is with the smallest capacity. The time of charge depends very largely on the dark period currents and not so much on the voltage range. These results obtained with a commercial lamp show that it is not suitable for use with a capacity of such a size. It is also very noticeable that there are large variations in the striking potential.

4.—*Effect of γ-ray irradiation on the tube.*

Prints 1 to 6 illustrate this point. Prints 1, 3, and 5 were obtained with the tube in the dark and capacities ·0005, ·005, and ·05 with various charging

currents, while 2, 4, and 6 were obtained under exactly similar conditions, except that a tube containing a few milligrams of radium was placed near the discharge tube. It will be seen that for the smallest capacity the effect is very marked, the presence of the radium greatly increasing the regularity of the flash and also reducing the voltage range and dark current. Prints 3 and 4 show the diminished effect with a larger capacity. There is still, however, an appreciable decrease in the range, and with it the maximum dark current. In curve B, Fig. 5, the values of the current for print 4 are shown. It will be seen that there is a small increase of the dark current produced by the radium in the early stages, but that, owing to the lowering of the striking voltage, the current never reaches so high a value. The flashing rate is increased by the latter two factors. For still larger capacities, as prints 5 and 6 show, the effect of the radium is hardly noticeable. The six prints also show how much more regular the flashing is with the larger capacities.

5.—*Effect of geometrical arrangement of electrodes.*

It was found that the shape and position of the electrodes are of the greatest importance. If the electrodes are not of the same size and shape interchanging the anode and cathode may entirely alter the behaviour of the tube. Prints 1 to 7 were obtained, as previously mentioned, with a commercial neon letter lamp. In this lamp one electrode is an L-shaped plate and the other a short cylindrical bar. In these seven records the plate was cathode, as the tube was found to be more reliable under these conditions. Prints 8 and 9 show the effect of using the rod as cathode. In print 8 the capacity is ·005 and the diode current 30×10^{-8} amp. This print may be compared with No. 3, using the same capacity and a diode current of $6{\cdot}8 \times 10^{-8}$ amp. The irregularity of flash is at once noticeable. The lag in striking due to increased dark current is very variable, and the striking voltage also shows large variations. It was found by direct visual readings that the average voltage range is slightly less with the bar as cathode. Print 9 shows the effect of radium. The change produced in the flashing is more striking, but of the same kind as with the plate as anode. The reduction in voltage range produced by the radium is much larger for the bar as cathode, and other records obtained confirm this result.

To investigate this point further special neon tubes were constructed. These were filled with a mixture of 80 per cent. neon and 20 per cent. helium, with probable traces of other gases. The electrodes were made of outgassed nickel, and various gas pressures ranging from 4 to 10 mm. were tried. In this range the behaviour of the tubes was found not to be very sensitive to pressure, but further work on this point is necessary. Print 10 is for a tube with concentric cylindrical electrodes of diameter 1 and 5 mm., respectively, the filling pressure being 3·9 mm. Two superimposed records are shown on the print, the slow large-range flash being for the outer cylinder as cathode and the quick short-

range with the inner cylinder as cathode. The diode current is the same in both cases. The bigger range for this tube was much larger than the commercial type, being between 65 and 70 volts. The average range for the inner cylinder as cathode is approximately 1/6th of this. This seems to show that it is the total variation of the electric field near the cathode that determines the flashing, as in this tube the field would be radial, neglecting space charge effects, and the value of the field near the inner electrode, therefore, five times that near the outer, which is about the same as the ratio of the voltage ranges.

Prints 11 and 12 illustrate this point further. These are for a three-lead tube with point, plane, and cylindrical electrodes. The plane is a disk of 1 cm. diameter, with the point about 1·5 mm. distant from its centre. The cylindrical electrode is 1·8 cm. in diameter and surrounds the other two electrodes completely. It is intended to act as a shield to the inner two electrodes, and is kept at some fixed potential intermediate to that of the other two electrodes. This potential was chosen so that the difference between it and either of the other electrodes is less than the extinction voltage of the tube, otherwise a continuous discharge will occur in the tube. The filling pressure was 5·5 mm. of Hg. Print 11 shows two runs of the oscillograph, the shunting capacity being 0·5 microfarad and the diode current 42×10^{-6} amp., the large range oscillation being with the plate as cathode, and the small with the point as cathode. The actual ranges are 47 and 12 volts. Print 12 is a similar record, with a shunting capacity of ·005 microfarad and a diode current $2{\cdot}0 \times 10^{-7}$ amp. In this the ranges are 42 and 18 volts. The beading noticeable in this record is due to some induced effect of the A.C. supply in the building, as it disappears if the supply is cut off at the main switch. With the plate as cathode, this tube is an exception to the general rule that the voltage range increases as the shunting capacity decreases, the range being slightly less for the smaller capacities.

The size of the electrodes also affects the change of resistance produced in the tube during the flash. When the smaller electrode is used as cathode the range is reduced, but, although a smaller quantity of electricity is transferred per flash, the duration of the flash may be nearly as great, owing to the smaller change in the resistance of the tube. Print 10 shows this effect slightly, and print 11 very clearly. With the point as cathode in this case, the charging period is only about double the discharge period, while with the plate as cathode the charging period is nearly six times the discharge period. For the other tubes the ratio is much larger than this. In general it appears that the behaviour of a tube is more satisfactory with the larger electrode as cathode, as, although this increases the range, the gain in regularity more than compensates for the resultant loss of sensitivity.

In conclusion, I must apologise for the omission of any theory of the various effects observed, but the flashing phenomenon is evidently so complicated and affected by so many factors that it is perhaps premature to attempt this yet.

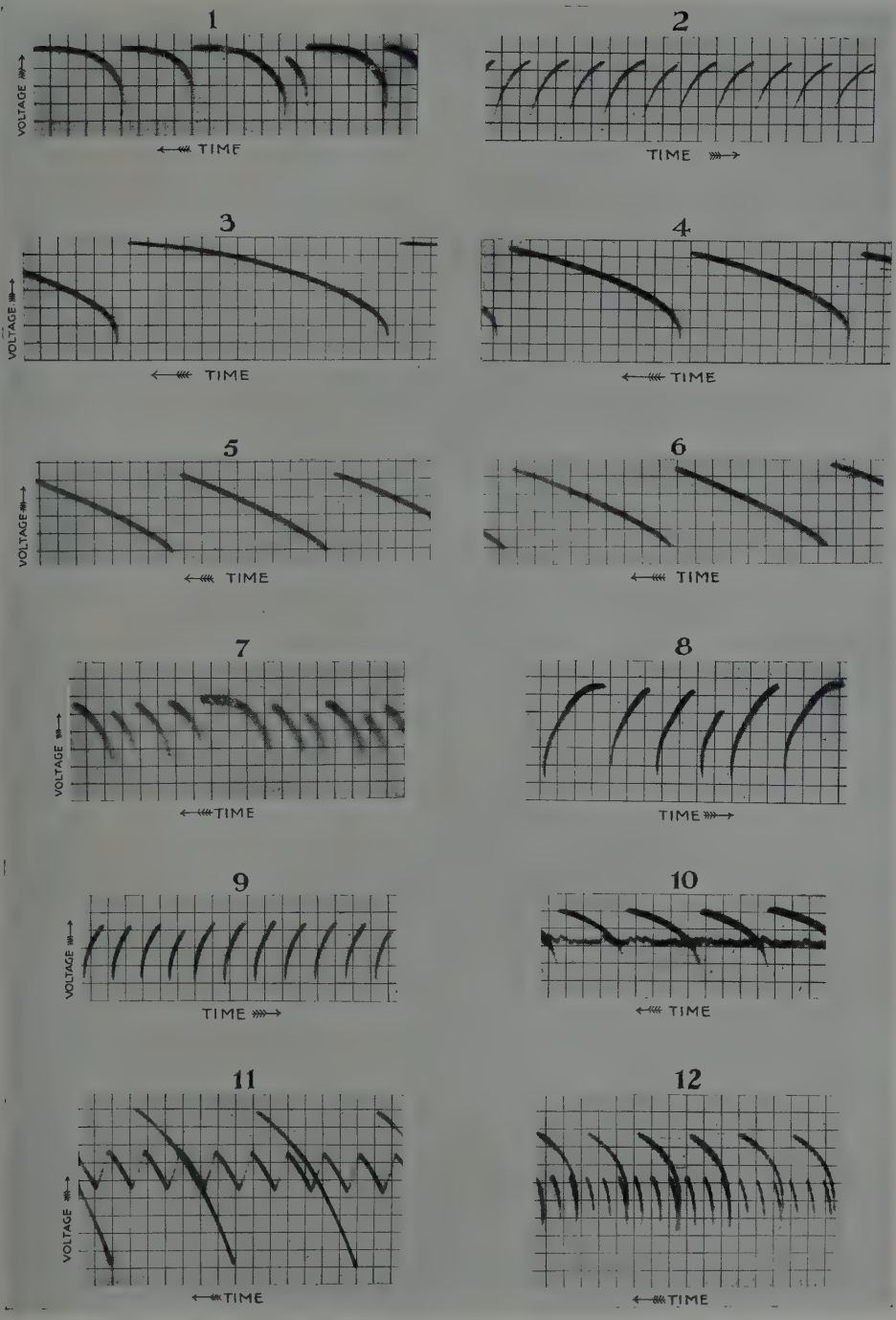
I should like to take this opportunity of acknowledging with thanks the

grant made by the Royal Dublin Society for the purchase of the oscillograph and its high tension batteries.

Plate 44.

Particulars of Prints.

Print Numbers.	Shunting Capacity.	Diode Current.	Time Scale 1 Unit.	Voltage Scale 1 Unit.
1 & 2	·0005 μF	$5{\cdot}0 \times 10^{-8}$ amp.	·30 sec.	7·3 volts.
3 & 4	·005 „	$6{\cdot}8 \times 10^{-8}$ „	·30 „	5 6 „
5 & 6	·05 „	$7{\cdot}8 \times 10^{-7}$ „	·30 „	7·3 „
7	·0005 „	$1{\cdot}2 \times 10^{-7}$ „	·30 „	7·3 „
8 & 9	·005 „	$3{\cdot}0 \times 10^{-7}$ „	·30 „	5·6 „
10	·0005 „	$2{\cdot}2 \times 10^{-7}$ „	·21 „	10·5 „
11	·5 „	$4{\cdot}2 \times 10^{-5}$ „	·11 „	5·3 „
12	·005 „	$2{\cdot}0 \times 10^{-7}$ „	·11 „	5·3 „


1
VOLTAGE
TIME
2
TIME
3
VOLTAGE
TIME
4
TIME
5
VOLTAGE
TIME
6
TIME
7
VOLTAGE
TIME
8
TIME
9
VOLTAGE
TIME
10
TIME
11
VOLTAGE
TIME
12
TIME

No. 33.

BAST-SAP.

By HENRY H. DIXON, Sc.D., F.R.S.,
University Professor of Botany in Trinity College, Dublin.

(Plate 45.)

[Read December 20, 1932. Published separately January 18, 1933.]

The problem of the transport of organic substances in plants has recently been the subject of much discussion and research. The work of Mason and Maskell (5, 6, 7, 8, 9, 10, 11, 12) and that of Münch (13) have shown that, although some of this transport may be effected through the wood (1), the major part is transmitted through channels in the inner bast. With regard to the mechanism of this transport in the bast, there is still much obscurity, and divergent views have been advocated. Mason and Maskell, relying on their results showing that the movement is, in almost all cases, from places of high tot places of low concentration, hold that the movement is analogous to diffusion. Münch on the other side considers that mass-movement of the entire contents of the sieve-tubes is produced by differences in osmotic pressure between the upper and lower regions of the bast system of plants.

In 1858 Th. Hartig (3 and 4) called attention to the fact that many trees, when their bark is punctured in summer and autumn, yield drops of clear sap. This sap was said to issue from the inner layers of the bast, and to include small quantities of nitrogenous substances, which could be coagulated by boiling; and, furthermore, he reported that this sap contained 25–33 per cent. of various kinds of sugar. Evidently examination of this sap might yield valuable information as to the form in which these substances are transmitted and on the method of their transport.

In the first place it may be noted that a puncture yielding this sap must be, as may be determined by subsequent microscopic examination, of such a depth that it reaches the inner bast, and does not open into the wood. If a puncture already exuding sap is slightly deepened (so as to reach the wood). the exuded sap on the surface of the bark is instantly drawn in by the tensile water in the tracheae. This is a striking demonstration that the exuded sap is not raised by root-pressure through the outer xylem.

It is possible to show that the movement of bast-sap takes place in the sieve-tubes. To effect this we may fix over an exuding puncture a small cylindrical gun-metal container (*C*, Fig. 1) held against the bark by an iron strap (*A*) round the stem, and making connection with the bark by means of a rubber washer (Fig. 2, *F*) coated with glue. A flexible lead tube (*E*, Fig. 2) connects the container with a suitable pump. When the container (*A*, Fig. 2)

and the glued washer (*F*, Fig. 2) are brought into position, the iron strap is tightened with a bolt (*B*, Fig. 1) and the container is filled through a screw bung (*B*, Fig. 2) with a 1 per cent. solution of potassium ferrocyanide. The bung is then screwed home and the pressure raised by means of the pump. After a certain time the pressure is relieved and the apparatus is removed from the stem; the bark is washed and a piece of it round the puncture and extending into the wood is excised. This portion may now be immersed in a solution of ferric chloride, and the penetration of the potassium ferrocyanide solution revealed by the presence of the precipitate examined macroscopically (Fig. 3), or the piece of bark may be sectioned and the sections examined microscopically in ferric chloride solution. The latter method shows clearly that the potassium ferrocyanide has been forced by the external pressure through the puncture into and along the sieve-tubes. The bast parenchyma and the medullary-ray-cells in contrast remain free from the precipitate.

The movement of water up or down in the sieve-tubes from the puncture may also be inferred from the behaviour of dilute Indian ink substituted for the potassium ferrocyanide solution. In this form of the experiment the colloidal ink is carried with the water moving into the opened sieve-tubes, and is only stopped when it comes up against the terminal wall of the tube (Fig. 4).

The bast-sap is easily collected from punctures made in the bark of various trees by the point of a small knife driven 2–5 mm. deep. It is advisable in most instances to clean the surface of the bark with vaseline. All specimens of the same species in the same locality do not exude bast-sap simultaneously. The most generous supplies seem to come from young trees 12–30 cm. in diameter. The flow is difficult to observe in trees with rough or papery bark. I have not seen it in Gymnosperms, with the doubtful exception of *Larix leptolepis*. It has also been noticed in various herbaceous plants. Among the 40–50 species which have shown it the following may be mentioned:—

Acer pseudoplatanus.
Acer saccharum.
Aralia mandshurica.
Azara dentata.
Aucuba japonica.
Betula alba.
Buxus sempervirens.
Cercis siliquastrum.
Colutea microphylla.
Cornus sanguinea.
Corylus avellana.
Crataegus oxyacantha.
Cytisus laburnum.
Cytisus vossii.
Davidia involucrata.
Escallonia rubra.
Eucalyptus globulus.
Fagus adiantifolia.
Fagus silvatica.
Ficus carica.
Fraxinus americana.
Fraxinus excelsior.
Heracleum sphondylium.
Ilex aquifolium.
Larix leptolepis.
Liriodendron tulipifera.
Magnolia wilsoni.
Mespilus communis.
Morus nigra.
Paulownia imperialis.
Populus nigra.
Ptelea trifoliata.
Pyrus communis.
Pyrus malus.
Pyrus pinnatifida.
Quercus robur.
Rhus typhina.
Rosa canina.
Rubus fruticosus.
Sambucus nigra.
Scrophularia nodosa.
Tilia americana.
Ulmus campestris.
Viburnum lentago.
Viburnum opulus.
Wistaria sinensis.

The exudation was first looked for and found on June 10th, and showed itself throughout the months of June, July, August, and September. In the case of an example of *Fraxinus excelsior*, which was under special observation, it continued up to October 20th, and could be detected even as late as October 27th. This tree had completely lost its leaves on October 17th.

The amounts exuded are variable. In active specimens the flow from a slit-like puncture 1 mm. long continues for 30–45 minutes, or longer; and during that time yields from 0·1–0·2 c.c. The lateral proximity of other slits does not, within limits, affect the amount of flow, and prolonged flow has been observed from 4 punctures vertically above one another 1 cm. apart. Active flow, however, from a puncture seems to reduce considerably, or even inhibit, the flow from punctures 0·5 cm. above or below. The amounts delivered from similar punctures at the same level, even when not influenced by neighbouring punctures, are not uniform. Thus when a ring of punctures was made, by a minute chisel 1 mm. wide, round an active stem, it was observed that the amounts exuded per puncture per minute varied from 0 to 0·041 c.c., and the regions of exuding bast were very erratically distributed round the circumference of the stem.

Measurements of the rates of exudation from individual punctures were made by timing the interval required to fill a capillary tube; the volume of this tube having first been determined by weighing it full of mercury and empty.

An examination of the exuded liquid confirms Hartig's statement as to the contents of bast-sap. Small quantities of proteins and glucose were found in it, associated with considerable amounts of sucrose—up to 12 per cent. In addition evidence for the presence of tannin, an oxidase, and a chromogen, was obtained. Evidently the whole phenomenon of the exudation of bast-sap suggests that the inner bast furnishes a channel suitable for the longitudinal mass-movement of organic substances under certain conditions.

This conclusion is confirmed by a further observation that, when exudation has ceased from an active puncture, renewal of the flow may be produced by exposing the upper leafy part of the stem to a gas pressure of 5–13 atmospheres.

These observations render it plain that the sap extruded from the punctured bark is moved by the turgor of a closed system in the bast (the sieve-tubes) and only ceases when that pressure becomes negligible.

An examination of cross-sections of the bast in *liq. iodi* makes the relations very clear. In them the sieve-tubes appear empty with re-entrant walls, while the cells of the bast parenchyma, loaded with starch, have bulging contours impinging on the sieve-tubes. A major limit of this turgor may be found by ascertaining the osmotic pressure of the sap exuded at higher levels from this system. With this aim determinations of the freezing-point of the sap were made by means of the thermo-electric method, and its osmotic pressure calculated. Unexpectedly high results were obtained, varying from 13–35 atm. Evidently the forces available for moving the sap through the bast and forcing it from the punctures are very high. A further point of great interest was observed: while very different osmotic pressures are observed in the bast-sap of the same

specimen on various occasions, it has, so far, always been found that sap drawn off at a higher level possesses a higher osmotic pressure than that simultaneously emerging at a lower level. In the following table the results of four such comparative experiments on a young specimen of *Fraxinus excelsior* are given. Here are recorded the dates on which the sap was collected, the height of the punctures above the ground, and, under $\triangle$ and P respectively, the depression of freezing-point and the osmotic pressure of the sap tapped off.

Bast Sap of Fraxinus excelsior.

Date.	Height	$\triangle$	P
Sept. 6	135 cm.	2·54°	30·6 atm.
„ 6	15 „	1·99°	24·0 „
„ 15	166 „	2·49°	30·0 „
„ 15	10 „	1·94°	23·4 „
„ 15	145 „	2·93°	35·3 „
„ 15	14 „	2·25°	27·1 „
„ 17	258 „	2·41°	29·0 „
„ 17	168 „	2·25°	27·1 „
„ 17	12 „	1·09°	13·1 „

It may be noted that the amount of sugar found in the sap does not account for the whole of the osmotic pressure (depression of freezing-point) observed, and it is interesting to speculate on the nature of the solutes present. The sap is practically neutral. There are insignificant amounts, if any, of nitrates and chlorides.

The rapidity with which the bast-sap is driven out of punctures indicates that, with the pressures available (13–35 atmospheres), it is readily mobile in the channels of the bast. The mobility, as one would expect from the longitudinal extension of the sieve-tubes, is chiefly in a longitudinal direction. This was illustrated in an experiment in which observations were made of the amounts delivered at uniform punctures uniformly distributed round a horizontal cut in the bark (Fig. 5). The cut which exposed the wood was made some two months previously. It was 4 cm. long, and 11 punctures were made along its upper and lower margins. It was observed that two of the punctures immediately

over the middle of the cut yielded no bast-sap, and the same was true of the 5 punctures occupying the middle region of the lower margin.

The limited lateral movement in the bast is also apparent in specimens injected with potassium ferrocyanide by the method described above. One of these is shown in Fig. 3. Here a strip of bark, viewed from the inside surface, is figured. It was removed from the tree after injection through the 3 punctures *a*, *b* and *c*. The line *d* indicates the mark of an old scar now completely healed. The darkened areas show the regions of the bast traversed by the solution of potassium ferrocyanide and brought into prominence when the strip was immersed in the solution of ferric chloride. The block caused by the old scar, as well as the small lateral spread, may be noted.

In experiments carried out on these lines it has been found that the solution of potassium ferrocyanide is moved in the bast under an external pressure of 3 atm. about 7–8 cm. per hour. Two factors, at least, tend to make this estimate of the conductivity of the sieve-tubes for the solution too low :—(1) The pressure exerted by the strap to keep the container in position probably crushed the sieve-tubes more or less, and so diminished their cross-section. (2) The slimy débris from the margin of the puncture probably clogged the entrance into the opened sieve-tubes, and so increased their resistance.

As we have seen, punctures 1 mm. wide deliver up to 0·04 c.c. per min. of bast sap. The thickness of the transmitting bast, containing 4–6 layers of soft bast, is about 1 mm. Of this cross-section (1 sq. mm. area) sieve-tubes do not contribute more than 0·2 sq. mm., if as much. Consequently the velocity of flow in the sieve-tubes during exudation must be at least 10 cm. per min.

Both these methods, depending (1) on the rate of injection, and (2) on the rate of exudation, indicate that the conductivity of the sieve-tubes for solutions is considerable, and hence we may assume that the sieve-tubes are available for the transport of organic substances.

The gradient of osmotic pressure observed in the bast-system is from 2 atm. per metre upwards. This gradient is probably maintained by the production of carbohydrates in the leaves, and by their removal by condensation into products of growth (activity of the cambium) and materials of storage (sucrose, starch, and fat, formed by the cells of the starch sheaths of the cortex and of the medullary rays). The difference in osmotic pressure above and below is available for forcing the solvent, water, of the less concentrated solutions below, into the wood, thus creating a mass-movement of the solution downwards through the bast.

The velocity of this mass-movement in the uninjured plant is not to be supposed to be at all as great as that observed in the exudation experiments, because of the opposition offered to the passage of water by the membranes enclosing the lower bast. The introduction of carbohydrates from the photo-synthetic cells, and the osmotic absorption of water from the tracheae in the leaves into the upper elements of the bast is able to maintain this mass-movement owing to the great surfaces available for these processes compared with the

small cross-section of the conducting bast. Similarly great surfaces are available in the bast of the stem and roots for the extraction of carbohydrates and for the passage of water from the sieve-tubes largely back into the tracheae, when the extraction has rendered the contained solution sufficiently dilute.

Thus we have obtained from several new points of view support for the theory which regards the bast as the most important channel of transport for organic substances, and supposes that the movement along this channel is maintained by the differences in the osmotic pressures obtaining in the upper and lower levels of the bast.

This theory offers adequate explanation for the various experimental results of Mason and Maskell:—(1) The increased concentration above a ring is due (*a*) to the downward movement of the concentrated sap-column allowed by the exudation from the wound and by the passage of water from the bast immediately above the ring into the wood, and (*b*) the retention of this concentrated column by the healing of the wound. (2) The diminished concentration below the ring receives its explanation according to this view from (*a*) the rise of the lower dilute sap from below owing to leakage into the wound, (*b*) the cutting off of supplies from above by the interruption caused by the ring in the channel of the bast, and (*c*) the continued depletion of the solutions in the lower bast by the adjacent cells in condensing their contents into substances less osmotically active. (3) The various forms of the flap-experiment yield results evidently explicable by, and confirmatory of, this theory. (4) Movement of carbohydrates along the concentration gradient also is, as has been pointed out, to be expected according to the theory; but it should be noted that, of the contents of the bast-sap, the sugars, owing to their very high concentration and osmotic activity, exercise a preponderating control of the direction of flow. This is in perfect agreement with Mason and Maskell's results, and supplies adequate explanation of the less regular results obtained for the much less concentrated and less osmotically active nitrogen compounds. In fact they are borne along passively without regard to their concentration in the carbohydrate stream. It may also be noted that while, so far as present observation goes, the carbohydrate solution moves down the concentration gradient, this need not necessarily always be so, and probably many exceptions will be found; for between the terminal osmotic pressures above and below in the bast-system reversed gradients in the stream, due to any cause whatever, need not cause any change in the direction of the mass-movement, which is determined almost entirely by the difference of osmotic pressure acting on the upper and lower semi-permeable membranes of the whole system. It may be noted, however, that the abstraction of solutes all along the path of transport will tend to reduce continuously the concentration of the descending sap, and maintain the gradient.

The recognition of the probable intervention of osmotic pressure differences as a motive force for the transport of substances through the sieve-tubes raises the possibility that a similar function should be attributed to various other tubular structures found in parenchymatous tissues, *e.g.*, the latex tubes of

various plants and the peculiar tubular elements in the vascular bundles of the haustoria of Lodoicea (2).

I desire to express my indebtedness to Mr. M. W. Gibbon for help in collecting the sap and in determining its constituents; also to Mr. S. H. Nunan for several observations on the late autumnal sap-flow.

REFERENCES.

1. DIXON, HENRY H.—The Transport of Organic Substances in Plants. Presidential Address, Section K, British Association, 1922. Notes from the School of Botany, 3, p. 207.

2. DIXON, HENRY H., and BALL, NIGEL G.—On the Channels of Transport from the Storage Organs of Seedlings. Proc. R.D.S., **17**, p. 185, 1925.

3. HARTIG, TH.—Ueber den Herbstsaft in den Holzpflanzen. Bot. Ztg., 1858, p. 369.

4. HARTIG, TH.—Ueber die Bewegung des Saftes in den Holzpflanzen. Bot. Ztg., 1861, p. 17.

5 and 6. MASON, T. G., and MASKELL, E. J.—Studies on the Transport of Carbohydrates in the Cotton Plant. I and II. Ann. of Bot., 1928, p. 189 and p. 571.

7, 8, 9, 10, 11. MASON, T. G., and MASKELL, E. J.—Studies on the Transport of Nitrogenous Substances in the Cotton Plant. I, II, III, IV, and V. 1929, p. 205 and p. 615, 1930, p. 1, p. 233, and p. 657.

12. MASON, T. G., and MASKELL, E. J.—Further studies on Transport in the Cotton Plant. Ann. of Bot., 1931, p. 126.

13. MÜNCH, ERNST.—Die Stoffbewegungen in der Pflanze. Jena, Gustav Fischer. 1930.

DESCRIPTION OF PLATE 45.

Fig. 1.—Transverse section of stem, showing the metal strap (*A*) with bolt (*B*) holding the container (*C*) against the bark.

Fig. 2.—Longitudinal section of gun-metal container (*A*) with lateral opening (*B*) for filling. (*C*), collar for tightening connection (*D*) with pump. (*E*), tube leading from air pump. (*F*), compound washer of fibre and hard rubber insertion to fit the curvature of stem.

Fig. 3.—Sketch of the inner aspect of a strip removed from the bark of *Fraxinus excelsior* after injection with a solution of potassium ferrocyanide through the punctures *a*, *b* and *c* and immersion in a solution of ferric chloride. The dark regions extending above and below the punctures show the longitudinal and lateral spread of the first solution in the inner bast and its arrest by an old scar *d*. × 4.

Fig. 4.—Microphotograph of radial section in the plane of a puncture which had been injected with Indian ink. The injected sieve-tubes appear as vertical dark streaks. In each the upward, or downward, movement of the colloidal ink is arrested by the terminal sieve-plates. × 70.

Fig. 5.—Sketch of an old cut in the bark laying bare the wood. On the upper and lower margins of the cut are shown the punctures (numbered 1–11) in the bark and opening into the bast. × 2.

FIG. 1.

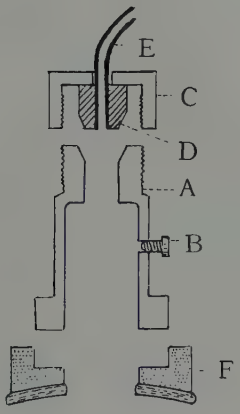


FIG. 2.

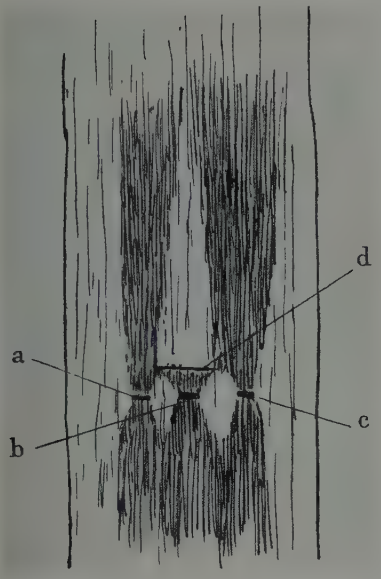


FIG. 3.

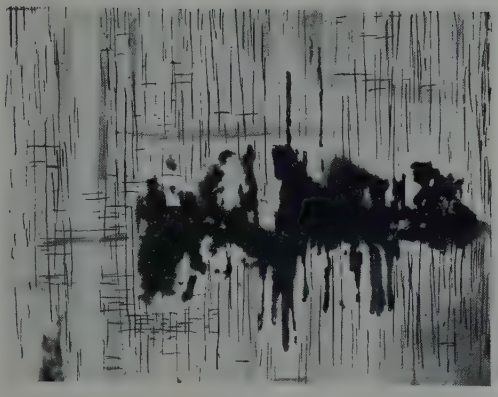


FIG. 4.

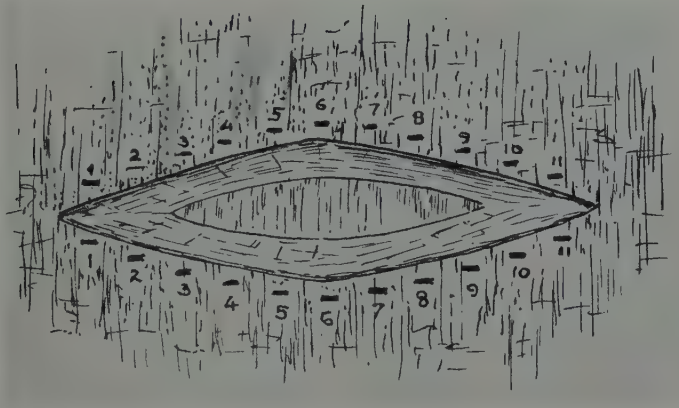


FIG. 5.

No. 34.

A STUDY OF *PHOMA LINGAM* (TODE) DESM., AND OF THE "DRY ROT" IT CAUSES, PARTICULARLY IN SWEDE TURNIPS.[1]

By WILLIAM HUGHES, B.Agr.Sc.,

Albert Agricultural College, Glasnevin, Dublin.

(Communicated by Dr. P. A. Murphy.)

(Plates 46 and 47.)

[Read June 26, 1932. Published separately February 20, 1933.]

The first technical reference to the occurrence of dry rot of swedes or turnips in Ireland appeared in 1903, when Johnson (11) recorded it as being widely spread throughout the country. He considered the disease similar to that previously recorded in England by Potter (19) in 1900. In Potter's account, which constituted the first exact record for England, the responsible fungus was not definitely named. Carruthers (4) in 1903 again described the disease and identified the parasite as *Phoma Napobrassicae* Rostr. (20), by which name it was known until 1927, when Cunningham (7) produced evidence to show that this fungus was synonymous with *Phoma Lingam* (Tode) Desm. The latter is now the accepted name.

Dry rot causes considerable loss in growing crops, and in storage, throughout the Irish Free State. Sometimes upwards of 50 per cent. of a crop may be infected during a bad season, and, as the disease occurs every year throughout the entire country, the total loss is considerable. Dry rot is also reported as prevalent in Great Britain (22), Denmark (2), and New Zealand (7). In the latter country its severity appears to be in excess of that usually encountered elsewhere.

In America, the same organism causes a serious disease of cabbages, Brussels sprouts, and cauliflowers, which is known as "black leg." This was shown to be seed-borne by Henderson (9), and a great deal of information has been secured concerning the life-history of the parasite, and its control on the plants mentioned.

The study of the disease caused by *Phoma Lingam* on swedes and turnips has been, by comparison, much neglected. Renewed impetus was given to this work by Cunningham's (7) investigations in New Zealand, and particularly by

[1] *Brassica napus* L. var. *napobrassica* (L.) Reichb.

his discovery that infection in the swede crop may also be contracted from the seed. In previous communications from this laboratory (13, 14), doubt has been thrown on the importance attributed in Cunningham's work to seed infection, as well as on the connection between *Phoma Lingam* and some of the fungi isolated from swede seed in New Zealand, and on the ability of the latter to cause dry rot. Under these circumstances it was decided to undertake a thorough investigation of the parasite, and of the disease it causes in swede turnips.

Incidentally, a certain amount of comparative work has been done on the forms of *Phoma Lingam* found associated with diseased conditions in cabbage (*Brassica oleracea*), charlock (*Sinapis arvensis*), and wallflower (*Cheiranthus Cheiri*). On the latter, serious losses have occurred in England (10).

The author wishes to acknowledge his indebtedness to Professor Murphy, under whose direction this work was carried out, and whose criticism and advice contributed largely to the success of the investigation.

Description of the Dry Rot Disease in Swede Turnips.

The disease may appear on the seedlings, and on the leaves, as well as on the fleshy roots, of older plants. The seed pods and seeds are also invaded sometimes. The description which follows is based on the appearances presented by plants grown under natural conditions, and constitutes the first full account of the development of the disease from the seedling stage onwards.

Seedlings.—The symptoms begin to appear seven to fourteen days after the plants come above ground, and the cotyledons are, as a rule, the first organs to be invaded. The attacked portion of the cotyledon assumes a duller shade of green, or a greyish colour, with an indefinite margin which merges imperceptibly into the normal tissue (fig. 1, Pl. 46). The spot enlarges rapidly, with the result that the whole cotyledon becomes dull in colour and shrivels up in two or three days. The disease may then progress into the other cotyledon and hypocotyl, following which the seedling usually collapses and dies. In the meantime abundant pycnidia are produced on the lesions, and these continue to appear after death ensues. More exceptionally, the hypocotyl is first invaded, due to contact between it and the seed coat below ground level, and the symptoms in this case are those of "damping-off."

Leaves.—The disease is not primarily one of the leaves, and little direct harm is done to these organs. It occurs most abundantly on leaves which are moribund or wilting (fig. 3, Pl. 46), and the spots are most evident on these because they remain green after the remainder of the leaf turns yellow. Lesions also occur, however, on leaves which are still vigorous (fig. 2, Pl. 46). As determined by spraying with a spore suspension, uninjured leaves are capable of being invaded, the symptoms beginning to appear after twelve to fourteen days. The first evidence of infection consists of the appearance of minute, depressed, pale green spots. These soon enlarge to 5 mm. in diameter, and

may eventually reach 20 mm., but this size is unusual, except at the edge of the leaf where they assume an irregular shape and a somewhat larger size. Elsewhere, they are circular at first, later somewhat irregular, dull green to ashy grey or light brown in colour, and they frequently become irregularly perforated in the centre. Pycnidia begin to appear three days after the spots are first visible. They are most abundant on the upper leaf surface, and are not arranged concentrically.

Fleshy Roots.—The appearance of dry rot on the roots is well known. The lesions are confined to the exposed surface, and are most abundant about the shoulder. They begin as small green spots, which soon become depressed and horizontally elongated, the centre meantime becoming brown or ashy grey in colour. When the root is young and growth is vigorous, a horizontal crack develops in the centre of the spot, and this may progress so far that the root is girdled and the top falls off, leaving a decaying stump in the ground. In most cases, however, an open gaping wound of variable size on the side of the root is characteristic of the disease (fig. 7–9, Pl. 46). In late attacks no cracking may develop.

Pycnidia are usually abundant, particularly after the spots crack open, or after the superficial portions of young green lesions have been eaten away by slugs (*Agriolimax* spp.), which have a partiality for this tissue.

In Storage.—Dry rot on stored roots is similar in appearance to the early stages of attack in the growing crop. Marked cracking, however, does not occur in the subsequent development of the lesions, and the latter remain for the most part superficial. Infections in the region of the neck, or on the cut surface where the roots have been trimmed off, penetrate the inner tissues more readily, and the decay is more rapid than that following infection on the sides.

Pycnidia are frequently produced, but under storage conditions the parasite does not usually fructify as freely as in field attacks, except in the case of those roots which become completely decayed.

Siliquas and Seed.—Lesions may occur on any portion of the surface of the siliqua, but are most common near the insertion of the stalk. They begin as small dark bluish spots, later enlarging rapidly and becoming ashy grey or light brown, with a depressed centre, on which pycnidia are produced. The siliqua is frequently distorted in consequence (fig. 5, Pl. 46). Within the siliqua there develops an abundant white mycelium which may envelop seeds situated a considerable distance from the externally visible lesion.

It has not been found possible to distinguish infected seeds by their appearance. Those formed directly under the lesions are light, shrivelled, and usually non-viable. Adhering fungus mycelium is visible on them, and *Phoma Lingam* occurs throughout their tissues, but such seeds are not believed to play any part in propagating the disease. Somewhat similarly shrivelled seeds sometimes occur in commercial seed samples, but *P. Lingam* has never been obtained from them, and they do not therefore necessarily, or usually, indicate the presence of dry rot infection.

The seeds which become contaminated without losing viability are apparently unchanged in appearance. Direct microscopic examination of seeds from infected siliquas has shown that a fungus mycelium, which was proved to be that of *P. Lingam,* is abundant in the three outer layers of the seed-coat[2]—namely, the disintegrating remains of the epidermal and cortical layers, and the columnar cells of the palisade layer, into the lumen of some of which it occasionally penetrates. The mycelium is, however, confined to these tissues, and no trace of it was found in the inner layers of the testa—namely, the remains of the coloured layer, the rectangular cells of the plasma layer with hyaline walls, and the internal layer. Sections cut with a freezing microtome and stained with methyl blue were used in this work.

A similar conclusion has been reached by dissecting away the seed-coats of presumably infected seeds, after preliminary steeping and superficial disinfection with mercuric chloride, and then plating out the coats and embryos separately on agar plates. The dry rot organism was secured from the testas on a number of occasions, but never from the interior of the seed, thus confirming the result of a similar experiment carried out by Cunningham (7) with swede seed. According to Orton and Rex (18), however, *Phoma Lingam* is not confined to any morphological unit of cabbage seed, but it is not stated if such completely infected seeds were viable. In cabbage seed, also, Nielsen (17) has observed the dormant mycelium occurring between the seed coat and the embryo, in the rudimentary seed albumen. Our observations indicate that invasion of the embryo, or of the soft developing seed, results in death. This is confirmed by the fact that the cotyledons of those seedlings which subsequently contract infection remain apparently free from the disease for about seven days after their emergence. The destruction is then so rapid that it is impossible to believe that the fungus can have been latent in them before that time.

Although it is clear that the fungus is confined to the outer layers of the testa of viable seeds, and is not normally able to penetrate through the mature seed-coat to the interior, indications were obtained that in the neighbourhood of the hilum, where the cells of the seed-coat are undifferentiated, penetration is sometimes possible, with the result that the embryo was in one case observed to be invaded at this point before germination.

The Relationship of Phoma Lingam *to Dry Rot.*

Very numerous isolation and inoculation experiments, followed by re-isolation of the parasite, have shown that *Phoma Lingam* is responsible for the disease symptoms described on cotyledons, leaves, fleshy roots, siliquas, and

[2] See Lund (12) for an account of the morphology of the seed of *Brassica oleracea, B. campestris* and *B. napus.*

seeds. It is not necessary to enter into the details of this work, which confirms the conclusions of a number of previous investigators.

The question of the relationship of *Phoma Lingam* to dry rot has been complicated by Cunningham's (7) discovery of strains of *Phoma,* which he includes in the same species. Cunningham consequently divided *Phoma Lingam* into three strains, according to their pathogenicity to swedes, and their rates of growth on potato glucose agar at 21° C., as follows:—

Strain I is weakly parasitic, and grows rapidly in culture, attaining a diameter of 75 mm. in five to ten days.

Strain II is strongly parasitic and slow-growing in culture, requiring eighteen to twenty-four days to attain a diameter of 75 mm.

Strain III is non-parasitic and rapid-growing in culture, attaining a diameter of 75 mm. in seven to ten days.

Strains I and II were further sub-divided, according to cultural characteristics, into groups designated I A, I B, and II A, II B.

In the main, Cunningham's conclusions in this connection have been verified in the present work. Fungi corresponding generally to his strains I, II, and III, and their sub-divisions, have also been met with, but it has been found that parasitic power cannot always be used as a basis for separating them. Thus, fungi have been isolated from swede seed which, although referable on all grounds other than parasitism to strain I B, proved strongly parasitic to non-growing swede roots in the laboratory. Strain I is, therefore, not homogeneous in respect of its parasitic powers. Again, the cultural characters used by Cunningham to separate strains I and II into A and B forms were found to be somewhat unreliable.

Considering the *Phoma* isolations referred to strain II in the present investigation, II A has always been identical in its cultural characters with the New Zealand strain II A. In II B, however, differences have been found. Cunningham's description states this to be slightly more rapid in growth than II A, and, in a graph showing the growth curves of the various strains, II B is indicated as attaining, on the average, a diameter of 75 mm. in seventeen days. In the present study, the cultures referred to strain II B have always been still more rapid in growth, attaining a diameter of 75 mm. in twelve to fourteen days. In other cultural characters, however, the II B isolated here appears to be identical with the New Zealand strain. In the subsequent discussion of strains in this paper, II B refers to the strain as isolated in the course of this work, as the slower growing form has not been found.

There is notable agreement, however, in the fact that the same form of the fungus has been found responsible for dry rot of swedes in Ireland and New Zealand, and that it falls into strain II. It has been found in the present

investigations that forms referable to strain II are invariably and exclusively associated with dry rot lesions on the fleshy roots, on the cotyledons, and on typical lesions on leaves which are not moribund. These are almost invariably II A forms, but not absolutely so, just as appears to be the case in New Zealand. Forms of *Phoma* corresponding to strains I and III occur on dying leaves, on roots showing other types of rot, and on the seed, but none of these fungi is capable of producing dry rot in the field, and the majority are merely saprophytes, a few being weakly parasitic. A summary is given in Table 1 of the classification, according to Cunningham's grouping, of all isolations of *Phoma* made during the course of this study. This table summarises work extending over a period of four years, during which more than 331 isolations of *Phoma* were made and studied.

TABLE 1.—Classification according to Cunningham's Grouping of all *Phoma* Isolations.

	No. of isolations falling into				
	Strain I		Strain II		Strain III
	A	B	A	B	
Swede cotyledons ...	0	0	35	0	0
Swede leaves	3	1	69	0	4
Swede roots	2	0	141	1	15
Swede seed	0	2	17	5	1
Turnip seed	0	1	0	0	0
Cabbage stems	0	0	0	24	0
Cabbage leaves	1	3	0	0	1
Wallflower stems ...	0	0	0	3	0
Charlock	0	0	0	2	0
TOTAL	6	7	262	35	21

Referring to the isolations from swede and turnip given in the upper portion of the table, it will be seen that strain II largely predominated over all others. Neglecting the saprophytic types included in strain III, which can have nothing to do with the production of dry rot, strain II was isolated in 142 cases out of 144 from dry rot lesions on the fleshy roots, in 35 cases out of 35 from cotyledons, in 69 cases out of 73 from leaf lesions, and in 22 cases

out of 25 from seeds. The conclusion seems evident that strain II alone is of practical moment in the production of dry rot. Where the somewhat parasitic forms of strain I occurred on the roots the symptoms were not typical of dry rot, and a bacterial disease was present, which appeared to be the principal cause of the decay. The same was true of all the isolations of the saprophytic forms of strain III, which always occurred in association with bacterial rot.

It is at least clear that strain II comprises the true, parasitic, *Phoma Lingam*. This has been divided by Cunningham into two sub-divisions, A and B. A includes a very slow-growing fungus with lobed colonies, which is strongly parasitic to swedes under all conditions. This is a homogeneous group, and indeed the only one of which this can be said. Strain II B is a very heterogeneous collection, judging by cultural characters, and all the forms which it has been found to include, with one exception, lack the power to produce dry rot in swedes under all conditions, in contrast to strain II A, though the forms belonging to both strains do so to an equal extent when inoculated into non-growing roots under the more favourable conditions of the laboratory. It will be noted that the II B strain has practically never been isolated from any portion of diseased swede plants with the exception of the seed, indicating that, so far as cotyledons, leaves, and roots are concerned, it is not an active parasite of these organs in nature.

It will be seen from the lower portion of Table 1 that strain II B is predominant on all the cruciferous plants examined, other than the swede.[3] This is at first sight surprising, because *Phoma Lingam*, as isolated by Walker from cabbages suffering from black-leg in the United States, was found here to belong to strain II A, and produced both in the laboratory and field the severe rot characteristic of this strain in swedes (Table 2). The American black-leg disease of cabbages is, if not entirely absent, certainly of no consequence in Ireland. The limited trials carried out here indicate that our typical *P. Lingam* from swede dry rot (corresponding to II A) causes little injury to cabbage stems under Irish conditions, and the provisional conclusion has been reached that the comparative absence of cabbage black-leg in Ireland is due rather to the conditions being unfavourable for this disease to develop than to the absence of the parasite. The disease of plants of the cabbage family which does occur, and with which fungi belonging to strain II B have been found associated, is apparently a less serious disease than black-leg.

Up to a certain point there are unmistakable indications that strains II A and II B of *Phoma Lingam* are at least specialised races—A adapted to an actively parasitic life on swedes (and possibly turnips), and B adapted to cabbages and other plants. Strong evidence in favour of this view is furnished in Table 1. The only portion of the swede plant from which II B forms have

[3] Too little work has been done on dry rot in turnips (*Brassica campestris* L. var. *rapa* (L.) Hartm.) to allow of the expression of an opinion as to whether II A or II B is likely to be the common fungus found in this crop.

been secured in any number is the seed. It is clear that the siliquas and seeds offer a suitable substratum to a variety of weakly parasitic or saprophytic fungi, as well as strongly parasitic ones, including, in addition to forms of *Phoma,* such fungi as *Alternaria* and *Macrosporium.* The occurrence of a *Phoma* on the seed, therefore, is no evidence of its active parasitism or its connection with dry rot.

A considerable amount of confirmatory evidence is furnished by inoculation experiments. When fungi belonging to strains II A and II B, and certain of those belonging to I B, are inoculated into wounds in healthy swede roots in a non-growing condition in the laboratory, all set up a dry rot, and no difference in symptoms or rate of development can be detected. When, however, the inoculations are made on the roots of similar plants growing in the field, infection is evidently more difficult; for, while the II A forms set up a progressive and typical dry rot almost without a case of failure, the I B and II B forms in question, with one notable exception in the case of the latter, have just as regularly failed to do so.

Observations made on swede roots inoculated in the field indicate that growing roots have definite recuperative powers which are not apparent in non-growing ones. Thus, in field inoculations, the wounds made in the control roots heal completely in less than one month, so that the only appearance of wounding is a surface depression. Non-growing roots when wounded do not heal in this manner. This recuperative property is, without doubt, responsible for the peculiar inoculation results referred to, since only the vigorous parasites are apparently capable of breaking down the natural resistance of the growing swede. This feature is especially apparent in the parasitic members of strain I, which produce a decay in non-growing roots in laboratory inoculations, while under field conditions they are incapable of forming a lesion.

The results of the field inoculations are shown in Table 2.

Were it not for the single case of isolation R 14 from a cabbage stem, which is not a II A form, but falls into II B, the evidence would be clear-cut, to the effect that the form of *Phoma Lingam* designated II A is exclusively associated with the dry rot of swedes, and that form II B is associated with a disease of cabbages and other cruciferous plants, but not of swedes. The fungus R 14, however, was isolated from a diseased cabbage stem, and resembled culturally all other forms found associated with the same disease on this plant, yet alone of these it proved strongly pathogenic to swedes in the field. Although it is not distinguishable morphologically from strain II A, it is quite distinct culturally. The fact of its occurrence makes it impossible to look on the strain II B as a specialised race of the species *P. Lingam* exclusively adapted to cabbages and other plants, while II A is similarly adapted to swedes, but there is a close approach to this condition.

There remains the question of the classification of the forms of *Phoma* included by Cunningham in his strains I and III. These differ from II A in their cultural characters somewhat more than II B does from the latter, but

TABLE 2.—Summary of Inoculations in the Field.

Year.	Strain.	Source.	Successful infection.
1928	I A	N.Z. 473	0/12
	"	N.Z. 503	0/12
	"	N.Z. 533	0/12
1929	"	N.Z. 473	0/12
	"	N.Z. 503	0/12
	"	N.Z. 533	0/12
1931	"	Swede roots (R 4)	0/6
	"	Cabbage leaf (L 58)	0/6
1928	I B	N.Z. 477	0/12
	"	N.Z. 505	0/12
1929	"	N.Z. 477	0/12
	"	N.Z. 505	0/12
	"	Swede seed (S 10)	0/12
1931	"	Cabbage leaf (L 57)	0/6
	"	do. (L 59)	0/6
	"	Swede leaf (L 69)	0/6
	"	Swede seed (S 10)	0/6
	"	do. (S 81)	0/6
	"	Turnip seed (S 80)	0/6
1928	II A	N.Z. 596	9/12
	"	N.Z. 491 B	12/12
	"	Swede root (A)	12/12
1929	"	N.Z. 596	11/12
	"	N.Z. 491 B	12/12
	"	Swede root (A)	12/12
1931	"	Swede root (A)	6/6
	"	Swede leaf (L 56)	6/6
	"	Swede seed (S 83)	6/6
	"	do. (S 84)	6/6
	"	P. Lingam (U.S.A.)	5/6
1931	II B	Swede seed (S 65)	0/6
	"	do. (S 82)	4/6 (1)
	"	Cabbage stem (R 10)	0/6
	"	do. (R 12)	0/6
	"	do. (R 13)	0/6
	"	do. (R 14)	4/6 (2)
	III	Swede roots (R 6, R 7, & R 8)	0/18
		P. oleracea—Holland	0/6
		Swede leaf (L 52, L 60)	0/12
1928		Controls	0/24
1929		"	0/24
1931		"	0/18

(1) Small lesions, not typical dry rot.
(2) The disease was indistinguishable from dry rot.

it has not been found possible to distinguish them morphologically. The occurrence of a form in strain I B, which, at least in laboratory inoculations, is equally as parasitic to swede roots as strain II B, coupled with the slight differences in cultural characters between the two strains, seems to force one to include strain I within the limits of the species *Phoma Lingam.* This classification is, however, regarded as provisional; for it would not be surprising to find that there are other accepted species of *Phoma* with which it comes into closer agreement. It has recently been noticed, for instance, that some forms included in strain I are morphologically closer to *Phoma destructiva* (Plowr.), C. O. Jamieson, than to *Phoma Lingam,* and, like the former species, they are capable of producing a rapid rot in tomato fruit. In order to settle this question a large amount of comparative morphological and cultural work with other species would be required, which is outside the scope of the present investigation. What is required, indeed, is a revision of the genus *Phoma,* a necessity to which the recent studies of Grimes, O'Connor, and Cummins (8) lend emphasis.

So far as strain III is concerned, the absence of morphological differences and its close similarity to many forms of strain I, some of which are only very weakly parasitic, make it undesirable at present to separate it from the latter, or to constitute a new species to contain it.

The Life-history of Phoma Lingam *and the Origin and Dissemination of Dry Rot.*

The disease is known to originate from one or more of three possible sources, namely—(1) the seed; (2) soil contaminated by the remnants of diseased roots from a previous crop which have been left exposed during the winter; and (3) similar remnants which reach the following turnip crop in farmyard manure.[4]

[4] The culture of the swede crop, as practised in Ireland, is as follows:—The rotation varies, but it is usually at least of four years' duration, swedes following a corn crop, or a crop of hay, or pasture following hay. It is unusual for two swede or turnip crops to follow consecutively, or at short intervals, on the same area, but consecutive crops may occupy adjacent portions of the same field. No other susceptible crop usually enters into the rotation. The land is almost invariably manured for the swede and turnip crop with farmyard manure, usually with the addition of at least phosphatic artificial manure. Occasionally artificial manure alone is used. The farmyard manure is applied in drills on the prepared ground, the drills are then closed, and the seed sown as soon as possible. Seeding may take place at any favourable time from early May to about the middle of June, or later in the case of turnips. If conditions are favourable, the seedlings appear in about one week, but they may be delayed for a month or more in dry weather. One month or more after sowing the plants are "thinned" or "singled" by hand, and the crop is subsequently hoed by hand once or twice. In these operations upwards of 96 per cent. of the total possible stand is removed and left lying on the surface of the ground; and the drills ultimately disappear, since the crests are hoed into the furrows. In addition, the crop is horse-hoed several times during the summer, from the time of "singling" until August. The crop is almost always pulled, "topped," and carted into the yard about December, where it is stored in straw-covered pits, and gradually fed to stock.

Infection Originating from Seed.—When infection originates from the seed, the cotyledons contract it while they are being pushed up through the soil, and while the seed-coat is still in contact with them. The symptoms may appear within 12 to 15 days after sowing, or one week or more after the seedlings emerge. The attacked cotyledon rapidly shrivels and dies, and, following this, the parasite passes down into the stem, and the whole seedling is invariably killed before any of the true, or "rough," leaves are formed. Fructifications begin to appear immediately, and secondary infections have been observed in experimental work in the open after a further period of 12 to 15 days, that is, 24 to 30 days after the seed was sown. This indicates that, even under the most favourable conditions for the development of the crop, secondary infection of the seedlings may take place before singling is done. In this operation the great majority of the plants are pulled up, and this number would include, in view of the very small number of infected seeds usually present, most of the seedlings which contract disease from this source, but this fact does not necessarily prevent such seedlings from serving as further sources of infection. It has been observed that the parasite fructifies freely on the dead or dying plants, and pycnidia containing viable spores have been found on the leaves of young plants removed in the operation of "singling" which had been lying exposed between the rows on the surface of the ground for six weeks. The carrying of such plants by tillage implements up and down the drills no doubt helps to distribute the disease.

While the foregoing is believed to represent accurately the course of development from about mid-May to mid-July, it must be emphasised that the disease is exceptionally difficult to detect during this period. This is due to the scarcity of primary infection centres, and not to any resistance on the part of young plants. Soon after mid-July, however, typical dry rot begins to appear on the developing fleshy roots when they are about 1·5 cm., or more, in diameter, and the attacked plants are conspicuous on account of the bluish-green colour of their leaves, and later by their wilted appearance. These roots are rapidly girdled and decayed, and the parasite fructifies very freely on them. From about the end of July onwards they serve as obvious infection centres, from which the disease spreads gradually to the immediately surrounding roots. Such centres remain visible, as a rule, until the end of the season, being marked by a completely decayed root in the centre, surrounded by a ring (or rather oval) of variable size, in which the roots show a gradually less advanced stage of decay as the periphery is approached. The extent of the spread along the length of the drills is always somewhat greater than across the drills (see plan, p. 519). It has been observed that the disease may spread slightly further to the north than in other directions, involving scattered roots beyond the inner infection centre. This is, no doubt, connected with the prevalence of southerly winds.

The dissemination of the disease is a slow one, and confined for a long time to a limited area, over which it spreads in a comparatively regular and

continuous manner. In severe attacks these areas may coalesce and overlap, but the disease never becomes epiphytotic over whole fields, as potato blight, for instance, generally does under the same conditions. These observations indicate that the splashing of rain, and to a much less extent the independent action of wind (22) apart from rain, is the principal agent of dispersal. The brushing of leaves together probably aids the spread to some degree, but most leaf infections during the late summer and autumn occur on the lowest leaves, which are very close to the ground, and are not readily capable of being brushed against neighbouring plants. On the other hand, the spread of disease up and down the drills is undoubtedly promoted by tillage implements, horses, men, and possibly other animals, such as rodents. The part played by slugs has already been mentioned.

Under certain exceptional conditions a more or less general infection of the younger upper foliage may take place in autumn. Such leaves have been found to be just as susceptible as the older leaves, and the reason why the disease is commoner on the latter is that they are nearer the sources of infection in the soil, or on the roots, and because of their proximity to the damp ground. The occasional general occurrence of leaf infection high up on the plants, while the roots remain for a time at least comparatively sound, is attributable probably to wind-borne spores from an outside source. Isolation from such leaves showed that the parasite was the typical dry rot organism.

Infection Originating from Soil.—The case of infection originating from the seed has been described first because the earliest outbreaks in the field (those on the cotyledons) are usually attributable to this source, but, as will be seen, the majority of attacks are not believed to originate in this way. The most serious outbreaks are known to occur where swedes are sown on fields which produced a diseased crop in the previous year. Sowing on sites on which the previous crop had been stored in pits is held to be a source of severe attacks in Denmark,[5] but no opportunity has presented itself for making observations on this point.

It has been established that *Phoma Lingam* is readily capable of surviving on partially rotted roots left on the surface of the ground under Irish conditions from December until the following July. These roots were found capable of initiating dry rot in the latter month, that is, after the elapse of seven months. This is a longer period than the parasite survives on similar roots under New Zealand conditions, where the disease is apparently more severe, and runs a quicker course. According to Cunningham (7), it is only in cases in which the first crop is fed off late and the second is sown early on the same land that the parasite survives in "mummied" roots. The question has not been determined whether *Phoma Lingam,* originating from the remains of such roots, can persist in the soil from one season to the second succeeding

[5] Information derived from Dr. E. Gram.

one, but this is not considered at all likely. The fact that the fungus can persist in certain types of soil under the artificial conditions of the laboratory is shown in a later section.

Under experimental conditions out-of-doors, infection of the cotyledons has been brought about in the entire absence of seed infection, the disease being contracted from the remains of a previously affected crop. The seedlings, however, were not attacked quite so early, nor in the same way, as in the case of seed-borne infection. The attack occurs when the cotyledons are beginning to yellow-off, and the invaded areas then appear as indefinite spots of a darker green colour, on which pycnidia are abundant. The parasite in this case has not been observed to pass down into the stem, or to cause damping-off. Such initially infected seedlings have been observed about five weeks after sowing, or about the time of singling. Thereafter the course of the disease is as already described, but more rapid.

The more rapid spread of the disease when contamination from a previous crop is present depends on a number of factors, including a (usually) much larger number of original sources of infection, and the fact that these are persistent and act over a longer period. It has been observed in such cases that the disease is most abundant on the lowest leaves, which hang down until almost in contact with the soil. The prostrate condition of the plants which remain after singling also favours infection in this case, because of direct contact with the soil, or with the remains of the superfluous plants in the furrow, on which the parasite may be abundant and active.

Infection Originating from Manure.—Dry rot may originate from farmyard manure contaminated with the remains of a previous diseased crop, and the resulting outbreak and course of the disease is generally similar to that arising from roots in the soil, though perhaps somewhat later in appearing.

Observation and experiment show that the later the diseased roots find their way to the manure the more dangerous they are to the ensuing crop. If added early in the year (prior to March) they appear to decay completely, and in experiments in which this was done they were not instrumental in originating the disease. The contrary was the case when they were added at the end of April. At this period of the year a great many roots, which have been rendered unfit for use by dry rot and bacterial soft rot, are invariably found on the site of the storage pits, mixed with the remains of the pit covering. It is this material, which, one may say, invariably reaches the manure, and usually at a period when all the tillage crops have been put in except swedes and turnips, that is the most dangerous source of new infections. The relative importance of infection originating from the seed, from soil, and from manure is discussed in a later section.

The Disease on Plants Grown for Seed.—It remains to describe the disease as it affects swede plants grown for a second year for the production of seed. Observations are based on comparatively large roots grown in the first year in the ordinary way for fodder purposes, and later transplanted into a special plot

in order to allow them to flower and seed. Such plants throw up flowering shoots which may reach a height of five feet, and probably attain a larger size than those grown for seed in the ordinary commercial way.

In the production of commercial seed, the nucleus stock of mother seed is produced by selected roots which are transplanted out of commercial crops for this purpose. The mother seed is then usually held over until the following summer, and is sown broadcast at the end of July or in August. The resulting plants, when about the thickness of a little finger, are transplanted into rows at any time from November onwards. They flower in this situation, and the seed is mature in August. The height the plants attain varies from about two to five feet, depending on the season. This fact may have a bearing on the extent to which seed infection follows, as the discussion below indicates.

Observations on diseased and healthy roots extending over three years show that when dry rot is present on the fleshy root of a plant intended for seed production, it continues to develop on the root as it stands in the ground during the winter, and may girdle it, and pass upwards into the neck. In spite of this, a flowering shoot is frequently produced, which usually falls over later under its own weight, and seed may or may not be matured, depending on the time the fracture takes place, and on its completeness. Infection of the siliquas and seeds is facilitated in the case of shoots which hang over in proximity to the diseased roots, and lesions have usually been found on the former under these circumstances.

In the case of plants of which the flowering shoots remain upright, observations on about 289 specimens showed that, although dry rot lesions were abundant on the roots, no trace of the disease could be found on the stem or leaves at a greater height than about 18 inches from the ground. The work was done in the period 1929 to 1931, seasons which for the most part might be considered favourable for dry rot. This negative result was unexpected and disappointing, since the experiment was set up principally for the production of seed carrying more infection than the traces present in most commercial samples. It was found that the only way in which such seed could be produced in any quantity under the existing conditions was by spraying the young seed pods with a spore suspension of *Phoma Lingam*. The highest proportion of infected seed secured in this way was 4 per cent. in the case of minute quantities of seed saved from individually selected diseased pods. The amount of infection in the bulk of the seed was, however, only 0·2 per cent. The experience of Neill and Brien in New Zealand is confirmatory (16).

Relative Importance of Infected Seed, Contaminated Soil, and Contaminated Manure as Sources of Infection.

A large amount of observational, as well as some experimental evidence to be given later, strongly suggests that seed infection, in the proportions in which it usually obtains, is not the principal cause of dry rot outbreaks in Ireland.

It was thought well to conduct an experiment at Glasnevin in 1931, under the most strictly controlled conditions possible, in which there would be approximately the same number of potential sources of infection, originating in the case of one plot from the seed, in another from the soil, and in the third from the manure, with a view to seeing how the disease would develop in each plot afterwards. Special precautions were taken to exclude all contamination from the manure, except that introduced in the form of rotting roots in measured quantity to one lot. The plots were isolated from each other in the same field, and the soil used was free from any possible contamination. Because, unfortunately, the seed selected for experimental work in that year was found on examination to show 3 infected seeds per 10,000, it was not possible to use disease-free seed for these plots (Table 3). The sample used, however, was disinfected beforehand, by heating it in water held at 50° C. for 25 minutes, as recommended by Clayton (6). Experience has shown that this treatment is reliable, and the behaviour of the control plot (No. 3) confirms this. Further evidence on this question is presented elsewhere. The seed for plots 4, 5, and 6 was not disinfected. The results of the experiment are shown in Table 3.

TABLE 3.—Spread of dry rot from seed, manure, and soil, approximately the same number of potential infection centres being present in each case.

No. of plot.	Variety.	Infection carried in.	Total length of drills in yards.	Number of potential infection centres per 100 yards.	Final percentage of dry rot.
1	Tipperary	Soil (1)	540	7	10·4
2	,,	Manure (2)	540	7	18·9
3	,,	(*Control*)	540	—	0·9
4	Swede 13/64	Seed (1/1000) (3)	220	10	0·9
5	"Special Swede" (4)	Seed (4/5000	1920	8	2·7
6	Turnip 6/77	Seed (1/1000)	220	10	0·0

(1) Roots showing dry rot dug into soil in previous December.
(2) Roots showing dry rot added to manure as it was being spread in the drills in May.
(3) This and the corresponding figures below show the number of infected seeds per 1,000.
(4) Seed kindly supplied by Mr. Buddin.

It is safe to conclude from this experiment that, under the conditions existing, seed infection was of practically no consequence, compared with infection arising from the same number of centres in the soil or manure. The amounts of infection in the seed sown in the last three plots were about those we have met in most average infected commercial samples. Data on this question relating to *Phoma Lingam* and other seed-borne fungi are given in the next section.

Extent of the Occurrence of Dry Rot Infection in Swede and Turnip Seeds, and its Significance.

During the course of this work, extending over four years, a total of 164,050 swede and turnip seeds, drawn from 42 commercial lots originating in Ireland and England, were mycologically examined for the presence of *Phoma Lingam*. A very large number of seeds of each lot was tested, varying as a rule from 1,000 up to 10,000, in view of the suggestion which has been made that exiguous amounts of seed infection were capable of setting up large amounts of disease in the resulting crop. Some of the lots were procured through ordinary commercial channels, while some were kindly supplied by seedsmen and others, as being likely to show a more than usual amount of disease. Due thanks are hereby tendered to all the firms and individuals who have helped us in this matter. One or two of these lots did prove to be unusually heavily infected, and in comparing one variety with another in the following table this should be borne in mind. As will be shown later, no evidence of a marked difference in the reaction of a number of common varieties of swede to dry rot has been secured. The samples are, therefore, believed to represent on the average at least the worst, as well possibly as the best, of the commercial seed on the market.

The examination was made by plating the seeds, after preliminary superficial disinfection in mercuric chloride 1 : 1,000 for ten minutes, followed by washing in sterile water, on two per cent. potato glucose agar, and incubating at 21 ° C. for seven days at least, following Cunningham's method (7) (fig. 6, Pl. 46). A mechanical seed counter and spacer was used, operated by suction, which greatly expedited the work. Every fungus originating from a seed was isolated and grown in pure culture, and in the very great majority of cases it was not discarded until fructifications were eventually secured. In the few remaining cases fructification could not be induced by any of the several means employed. Every culture of *Phoma* was single-spored in addition. A summary of the results is given in Table 4 below.

Of the total of 164,050 seeds of swede and turnip examined, the following numbers carried the fungi specified below :—

Phoma Lingam	...	26, or 1	infected	seed	in	6,310
Alternaria spp.	...	1,093, or 1	„	„	„	150
Macrosporium spp.	...	275, or 1	„	„	„	597

Small as the amount of infection is with *Phoma Lingam*, amounting on the average to 1 seed in 6,310, it is further to be observed that more than half of the total number of colonies of this fungus isolated came from two samples of seed, one of which was not from a commercial source, and both of which were

TABLE 4.—Results of the Mycological Examination of Swede and Turnip Seeds, 1928 to 1931.

Year.	Variety.	No. of seeds examined.	No. of seeds yielding cultures of— *Phoma Lingam.*	*Alternaria* spp.	*Macrosporium* spp.	Other fungi.
1928	Best-of-All (1) ...	500	0	5	1	0
	Caledonian ...	4,650	1	18	15	0
	Incomparable ...	500	0	0	0	0
	Magnificent ...	500	0	6	3	0
	Tipperary ...	5,100	0	27	15	1
	Up-to-Date ...	5,300	0	2	2	0
1929	Superlative 25 ...	5,000	0	6	28	1
	,, 26 ...	5,000	0	0	0	10
	,, 28 ...	5,000	10	3	0	0
	,, 60 ...	5,000	0	2	4	0
	Best-of-All (2) ...	5,000	0	14	4	0
	Caledonian ...	5,000	0	53	2	0
	Incomparable ...	5,000	0	2	6	0
	Magnificent ...	5,000	1	23	0	0
	Tipperary ...	5,000	0	14	1	0
	Up-to-Date ...	5,000	0	33	8	0
1930	Fulcrop 1. ...	5,000	1	79	15	0
	,, 2. ...	5,000	0	47	17	0
	Superlative (3) ..	10,000	0	117	27	0
1931	Superlative 93 ...	5,000	0	42	13	0
	,, 97 ...	5,000	0	28	9	0
1931	Greentop 106 ...	5,000	0	23	12	0
	Magnificent ...	5,000	0	15	4	0
	,, ...	5,000	0	9	7	0
	"Special" ...	5,000	4	5	2	2
	Turnip 6/75 ...	1,000	0	2	0	0
	,, 6/77 ...	1,000	1	107	11	0
	,, 7/74 ...	1,000	0	3	0	0
	Swede 9/69 ...	1,000	0	134	14	0
	,, 13/64 ...	1,000	1	49	11	0
	,, 8/55 ...	1,000	0	0	0	4
	,, 11/53 ...	1,000	0	2	0	0
	Tipperary (4) ...	10,000	3	0	0	3
	Greentop (5) ...	2,000	0	0	0	0
	Incomparable ...	2,000	3	19	3	0
	Magnificent ...	2,000	0	29	6	0
	Magnum Bonum ...	2,000	0	20	3	0
	Tipperary ...	2,000	0	0	0	0
	Knockadon (6) ...	5,000	0	0	0	0
	Magnificent ...	5,000	1	120	26	4
	Majestic ...	5,000	0	33	6	0
	Tipperary ...	5,000	0	2	0	3

(1) This and the following five varieties were used in the 1928 field experiments an account of which has already been given (14).

(2) This and the following five varieties were used in the 1929 field experiments (q.v.).

(3) Seed used in 1930 field experiments (q.v.).

(4) ,, ,, ,, 1931 ,, ,, (q.v.).

(5) This and the following four varieties were used in the Mountmellick experiment, 1931 (q.v.).

(6) This and the following three varieties were used in the Clare experiment, 1931 (q.v.).

supplied as being likely to show an unusual amount of the dry rot organism. It these abnormal lots are excluded, the average infection amounts to 1 seed in 12,837. It is quite clear that such an amount of infection is totally incapable of originating any material part of the amount of dry rot which develops year after year in practically every crop throughout the country.

The possibility was considered that the other fungi found on the seed might be parasitic. Inoculation experiments, however, have clearly shown that the *Alternaria* and *Macrosporium* isolations are non-parasitic on swede roots. No evidence whatever was obtained that any of the isolations had an *Alternaria*, or other spore-form in addition to a *Phoma* form. It has already been shown that neither the *Alternaria* nor *Macrosporium* is connected with *Phoma Lingam* (13).

Because of the difficulty experienced in finding *Phoma Lingam* on swede seeds, the possibility was considered that the technique employed in plating the seeds might be ill-adapted to its purpose. No evidence in support of this view was, however, obtained. So far as the preliminary superficial disinfection in mercuric chloride 1 : 1,000 is concerned, the period of immersion was varied with interesting results, as shown below. In this experiment a known heavily infected sample of swede seed, produced by the method already described, was used in lots of 300 seeds.

Duration of disinfection in minutes	No. of seeds producing colonies of:—		
	Phoma	*Alternaria*	*Macrosporium*
1	4	0	1
4	6	3	4
7	8	0	2
10	8	5	1

Increasing the period of disinfection up to 7 or 10 minutes caused an apparent increase in the number of *Phoma* colonies, due to the fact that when the period was shorter the plates became over-run with bacteria and common moulds, such as *Penicillium*, before the presence of the *Phoma* could be detected.

In another experiment similar heavily infected seed was divided into three uniform lots of 500 seeds each, one being plated in the usual way, another sown in sterile soil in the open under conditions favourable for dry rot, and the third germinated on moist filter paper in order to observe the viability of the seed. The results of this experiment are shown below, the records being taken separately for each lot of 100 seeds:—

Lots of 100 seeds	Germination in— Laboratory	Soil	No. of seeds producing *Phoma*— On Agar	On seedlings
1	88	83	5	3
2	86	90	5	3
3	89	85	4	4
4	88	81	3	3
5	92	92	5	2
Average p.c.	88	86	4·4	3

It will be seen that more *Phoma* colonies, in the aggregate, were produced on agar than became visible on the seedlings, indicating that the plating method gives a truer picture of the state of seed infection than does raising seedlings, or than any other method which has been devised. It is also more expeditious and convenient.

It is probable that the apparent failure of the full number of infected seedlings to appear, corresponding to the number of infected seeds, is due to the death or weakness of some of the seeds. Observation has shown that if the parasite penetrates into the embryo the seed is killed, and it is probable that in a case like this a proportion, at least, of the weak or dead seeds contain the dry rot organism. Such seeds would normally be expected to give rise to colonies of *P. Lingam* on agar plates. It is considered likely that infected seeds which fail to produce seedlings in the field are of no importance in initiating dry rot, although, as will be shown elsewhere in this paper, there is a theoretical possibility that the fungus might grow up to the surface through the soil, since it is able to live in sterilized soil which is rich in humus for a considerable period in the laboratory.

Confirmatory evidence of the reliability of the seed-plating method employed is furnished by other experiments. It has been a common experience during the course of this work that when seed which has been found in the laboratory to be free from dry rot infection is sown in large scale experimental work under ordinary farm conditions, serious amounts of dry rot develop at times in the resulting crop, even though steps are taken to exclude, as far as this is possible on the average farm, all other known sources of infection. Several instances of this occurred in 1928, and at that time they were considered to throw doubt on the reliability of the examination of the seed. Accordingly small plots were sown with the same seed, consisting of five lots, under more strictly controlled conditions at Glasnevin in 1929. The result was that no seedling infection whatever developed, and all the plots remained entirely free from dry rot until the close of the season, with the exception of one in which 4 roots in the outside drill developed dry rot at the end of September. This drill was separated only by a path from another plot containing diseased roots,

and the infection was clearly traceable to these. Under the same conditions, and in an adjacent plot, heavily infected seed similarly held over from 1928 produced diseased seedlings in 1929, from which dry rot spread to the surrounding roots.

It will be convenient to mention here an experiment in which the effectiveness was tested of Walker's method (21) as modified by Clayton (6) for treating infected cabbage seed, by steeping it in water held at 50° C. for 25 minutes. Portion of a very heavily infected sample of swede seed (produced by spraying the flowering plants with a spore-suspension of *Phoma Lingam*) was divided into two lots, one of which was treated by Clayton's method, while the other remained untreated. One thousand seeds of each lot were then plated out on potato glucose agar, with the following results :—

1,000 untreated seeds produced 20 *Phoma* colonies.
1,000 treated „ „ 0 „ „

This method of disinfecting swede seed is apparently fully effective, a conclusion which has also been reached as a result of field experiments in which it was used.

Saprophytic Growth of Phoma Lingam *in Soil.*

When known infected seeds, selected from beneath lesions caused by *Phoma Lingam* on the surface of seed pods, were sown in sterilized potting soil, mycelium, which was proved to be that of the dry rot organism, appeared on the surface of the soil over every seed within 6 to 10 days. No such mycelium appeared over non-infected seeds, and normal healthy plants were produced in this case. The seedlings from the infected seeds "damped-off" under moist conditions within two weeks, and *Phoma Lingam* fructified abundantly on them, and was isolated from them. The mycelium of this fungus also occurred abundantly in the surrounding soil, and on it pycnidia were also produced, sometimes in association with fragments of leaf-mould and sometimes not. The fungus was re-isolated both from this mycelium and from the pycnidia in the soil. Several repetitions of this experiment gave the same result.

Arising out of this, cultures of the fungus, originating from pure cultures on agar, and from roots showing dry rot, were established in similar sterilised potting soil in flasks. *Phoma Lingam* soon completely permeated the soil and produced pycnidia in it, and after ten days it was successfully transferred by means of the soil to other similar flasks. The same process was repeated in these, and the cultures were kept going in this way for six months. Platings on agar at intervals proved the presence of the fungus.

Attempts to grow the fungus in ordinary field soil which had been sterilized met with no permanent success. Weak growth was made at first, but no fructifications developed, and the fungus soon died out. Complete failure

attended efforts to establish cultures in unsterilized field soil, or to recover it from this medium. Under these circumstances the continued growth of the dry rot organism under experimental conditions in sterile soil very rich in humus is not believed to possess much practical importance under ordinary farm conditions. Nevertheless the fact that it can survive in any soil is interesting, indicating as it does the possession by the fungus of saprophytic possibilities.

Field Experiments concerning the Origin of Dry Rot, 1928–1931.

A number of field experiments were conducted during the seasons 1928 to 1931, inclusive, both at Glasnevin and, in co-operation with the Department of Agriculture, at the Agricultural Stations at Athenry and Clonakilty. Additional experiments were conducted in 1931 in Leix and Clare, in co-operation with the County Instructors in Agriculture. Acknowledgments are made for the willing assistance afforded throughout by the Department of Agriculture and its officers, especially Mr. R. Collins, Superintendent, Agricultural Station, Athenry, Mr. J. J. Hassett, Superintendent, Agricultural Station, Clonakilty, and by Mr. T. Tynan and Mr. W. Gahan, the Instructors in Agriculture in Leix and Clare, respectively.

The first field experiments carried out at Athenry and Clonakilty in 1928 were intended to establish if possible a relationship between seed infection and the subsequent development of dry rot, all other sources of infection being as far as possible excluded. Seed of six selected varieties was procured from the original raiser (with a view to greater uniformity), and this was mycologically examined for the presence of *Phoma Lingam*. The seed was sown on six uniform plots, which had an area of one-half acre each, at Athenry and Clonakilty, as well as on smaller plots at Glasnevin.

The experiment of 1928, of which an account has already appeared (14), suggested that seed infection was an important source of disease, for Caledonian, the only variety out of six in which the presence of the dry rot organism was established on the seed (1 seed in 4,650), showed by far the most dry rot at each of three widely separated centres. In the report referred to above the arguments for and against this view are set out, and the matter was left for confirmation or otherwise in further work. While it is difficult to escape from the conclusion that infection of the seed, small as it was in amount, must have been responsible for the uniformly preponderant amount of disease in Caledonian, it may be said at once that, if this was so, it is the only instance in which it was the case. In all subsequent experiments, without exception, no evidence can be discovered that seed-borne infection, even in considerably larger amounts, materially influenced the results.

Another fact has since come to light which also throws doubt on the part played by seed infection in Caledonian. The species of *Phoma* isolated from this seed belongs to Cunningham's group I B; it is not the *Phoma* II A, which

is nearly always associated with dry rot in the roots in Ireland. No extensive series of isolations were made from the Caledonian roots which developed dry rot in this experiment, but *Phoma Lingam* II A was secured in two cases from leaf lesions and in three cases from dry rot on roots, while I B was not recovered in any case. This shows that, despite the absence of II A from the seed, it was the principal cause of dry rot in the field. Furthermore, *Phoma* I B from this Caledonian seed (in common with other similar I B forms) has uniformly failed during each of three seasons to set up dry rot in the field, while the II A forms have regularly done so under the same conditions. Under laboratory conditions, however, the Caledonian I B has caused a decay similar to dry rot.

Experiments at Athenry and Clonakilty, 1929. Seed Infection.—The experiment of 1928 was repeated without change in the following year. Seed of the same six varieties, procured from the same sources, was mycologically examined, and one half of each lot was sown at Athenry and the other half at Clonakilty, under conditions believed to exclude infection from other sources. Each individual plot had an area of half an acre at both Stations. The results of the experiment of 1929 are shown in the following table (Table 5):—

TABLE 5.—Field experiments in 1929 at Athenry and Clonakilty.

Variety.	No. of infected seeds.	Per cent. dry rot by count.		Yield in tons per acre.			
				Athenry.		Clonakilty.	
		Athenry.	Clonakilty.	Healthy.	Diseased.	Healthy.	Diseased.
Best-of-All ...	0/5000	1·6	9·1	16·2	3·0	26·1	8·4
Caledonian ...	0/5000	9·5	6·6	16·7	2·7	25·5	11·1
Incomparable ...	0/5000	7·2	9·3	18·2	1·8	25·3	8·4
Magnificent ...	1/5000	5·5	12·0	18·0	2·0	30·1	6·4
Tipperary ...	0/5000	7·0	14·1	18·5	1·4	32·2	6·4
Up-to-Date ...	0/5000	5·0	11·6	20·1	2·5	28·2	9·4

Magnificent was the only variety to show any seed infection in the 5,000 seeds examined, yet it was in no way outstanding when the final percentage of dry rot in the crop was determined. These percentages illustrate a number of recurrent features in many duplicate large-scale experiments, to be described later. In the first place, dry rot is frequently considerably more common at one Station than the other, and, so far as the individual Stations are concerned, it tends to occur more or less equally in all varieties, irrespective of the condition of the seed. The first feature is known in certain cases to be explicable, in part at least, by different dates of sowing, or other similar factors; but the second appears to require the presence of generalised sources of infection,

other than the seed, which affect all the varieties more or less equally. If these sources were more abundant at one Station, they would help to account for dry rot being more prevalent there.

Experiments in 1930. Contamination in Manure and Soil.—The growing volume of evidence that seed infection was not the principal source of outbreaks led to a new series of experiments in 1930 and 1931, in which the effect of contamination in the manure and soil was studied. While swedes or turnips are not often grown consecutively, or at very short intervals, on the same area, successive crops are frequently taken in the same field on areas which may be contiguous, or may even overlap, particularly on small farms. On the other hand, there are few, if any, farms in the country on which swedes or turnips rendered unfit for use by dry rot do not reach the manure heap.

The 1930 experiments in this connection, which dealt with manure alone, were extremely carefully conducted, but on account of the fact that the results were largely negative, it is not necessary to detail them. All the plots showed a minimum of dry rot, there being only 3·8 and 1·4 per cent., respectively, in the contaminated manure plots at Athenry and Clonakilty, and 3·1 and 1·1 per cent. on the corresponding clean manure plots. In other words, the crops were nearly disease-free, for even of the attacked roots 90 per cent. were usable. The disease had appeared late, and was confined to individual isolated specimens, or small groups of two to four, in contrast with the large groups of 40 or more roots characteristic of early outbreaks. The manure was collected specially from the stables and piggeries, where no roots were being fed, and was stored in two separate heaps in the field, apart from the general bulk. To this fact is attributable the general absence of disease. One of the heaps was contaminated by having dug into it roots decaying with dry rot, this being done in March. Experience has since shown that if the roots had not been added until late in April or early in May a very different result would have been expected, since when added early they rot completely away and become harmless. In addition, the seed used (variety Superlative) showed no infected seed in 10,000.

A similar experiment on a smaller scale was conducted with the same seed and on the same general lines at Glasnevin in 1930, but with the addition of a contaminated soil plot, roots showing dry rot having been dug into the soil in the previous December. An equal number of similar roots were added to one-third of the manure in March, and this was applied to a second plot, similar, but uncontaminated, manure being used for the other two plots. The final results were as follows:—

Manure and soil clean.—1·6 per cent. dry rot in the crop.

Manure contaminated (*March*), *soil clean.*—1·2 per cent. dry rot in the crop.

Soil contaminated (*Dec.*), *manure clean.*—14·5 per cent. dry rot in the crop.

As in the two corresponding experiments in that year, the addition of decaying roots to the manure in March was without effect, but the infection persisted on decaying roots in the soil from December until the following summer, and led to a serious outbreak of disease.

Experiments in 1931. Contamination in Manure and Soil.—These experiments were repeated in 1931 at Glasnevin, Athenry, and Clonakilty, without material modification. The seed selected for the work, however, was found on examination to show 3 infected seeds in 10,000, and time did not permit of another lot being examined and substituted. All the seed, therefore, for the Glasnevin experiment and half of that for Athenry and Clonakilty was treated by Walker's method (21), as modified by Clayton (6), in hot water held at 50° C. for 25 minutes, which experience has shown to be entirely effective (see p. 514). The variety used was Tipperary.

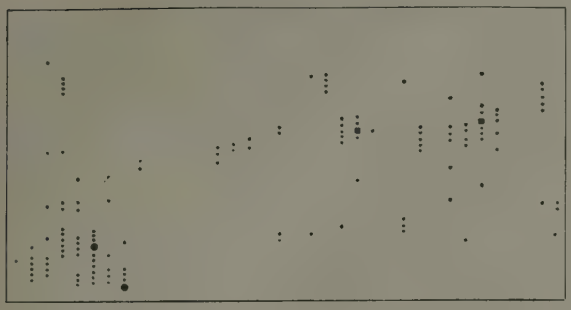
It will be convenient to give, first, the results of the Glasnevin experiment, which included a comparison of equal amounts of contamination in the soil and in the manure, the latter being added in May. The plots used had an area of 1/12 acre each. All possible precautions were taken as before to exclude infection from other sources. The final results of the experiment were as follows (compare plan opposite):—

Manure and soil clean.—0·9 per cent. dry rot in the crop.

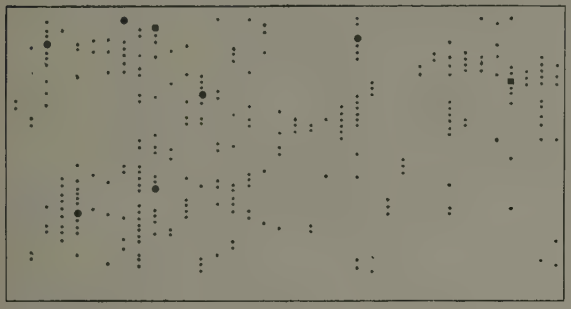
Manure contaminated (*May*), *soil clean.*—18·9 per cent. dry rot in the crop.

Soil contaminated (*Dec.*), *manure clean.*—10·4 per cent. dry rot in the crop.

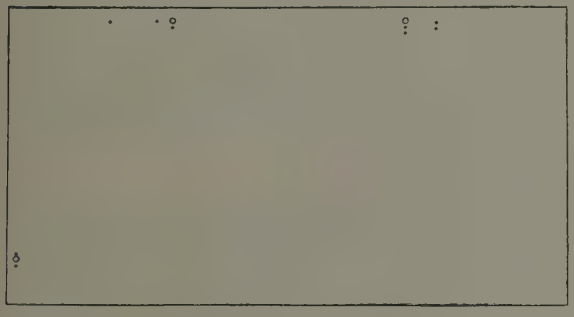
It is evident that the treatment of the seed was fully effective, since only 0·9 per cent. of dry rot developed in the control plot up to the close of the season. This did not appear until November, and then occurred on the plants most exposed to infection from the contaminated manure plot. The fact that the disease did not appear until November in the control plot while it was present from July in the contaminated soil and contaminated manure plots shows definitely that dry rot did not originate from latent sources in the seed or from the non-contaminated soil or manure of the control plot. The plan, which shows the dates of appearance and situations of the various primary outbreaks in the plots, and the subsequent spread of the disease from these centres, may be consulted in this connection. It is obvious that all the disease in the upper portion, at least, of the control plot was contracted from the plot above it. Rats were plentiful in the late autumn among the roots, as evidenced by their feeding on them, and they are believed to have helped in the spread of the disease.



Contaminated soil.



Contaminated manure.



Clean soil and manure.

PLAN OF FIELD EXPERIMENT IN 1931 COMPARING EFFECT OF CONTAMINATION IN SOIL AND IN MANURE.

Each small black dot represents a swede root showing dry rot.

The large black circles indicate roots which served as primary sources of infection in July.

The squares represent roots which served as primary sources of infection in August.

The open circles show roots forming local infection centres which did not become affected until November.

A distance of 25 yards containing oats separated the upper and middle plots, and one of 15 yards containing potatoes separated the middle and lower plots.

The large-scale experiments in 1931 gave a much less decisive result. This is a common feature of these experiments, and it arises, no doubt, from the greater difficulty of excluding all accidental contamination when operations are conducted on a scale comparable to that obtaining on the farm. The plots in these experiments had an area of one acre each, making two acres in all at each Station. The general conduct of the work was as in previous years, and the final results are shown in the following table:—

TABLE 6.—Field experiments in 1931 at Athenry and Clonakilty.

Seed.	Manure.	Per cent. dry rot by count.		Yield in tons per acre.			
				Athenry.		Clonakilty.	
		Athenry.	Clonakilty.	Healthy.	Diseased.	Healthy.	Diseased.
Treated	Clean	15·8	0·0	14·8	9·7	27·9	0·7
,,	Contaminated	34·4	0·4	9·6	14·6	25·7	1·2
Untreated	Clean	12·8	0·0	16·0	5·3	27·7	0·9
,,	Contaminated	25·7	0·3	11·1	14·0	28·4	0·6

All the plots at Clonakilty remained practically free from dry rot throughout the season, in spite of the presence of contamination in the manure in two plots, and in the seed in two cases. This is attributable to very late sowing, this operation having been delayed by the weather until June 19th, compared with May 3rd at Athenry. It is generally held throughout the country that late sowing reduces dry rot. The comparatively high yield obtained in spite of the delay in sowing is to be noted, but in a dry summer the crop might have failed completely owing to drought.

So far as the Athenry experiment is concerned, it is evident that there were generalised sources of infection throughout all the plots, although the use of contaminated manure more than doubled the amount of disease. The general infection cannot be attributed to the seed, for it cropped up equally where treated and untreated seed was used. The seed was the same as that sown at Glasnevin in 1931, both being disinfected at the same time, and there the evidence for the effectiveness of the treatment was convincing. The same elaborate precautions were taken as in 1930 to secure uncontaminated manure, and, so far as is known, the sources of infection could not have been introduced in this medium. The manure used was produced by pigs fed on meals and running on grass, and it was stored separately. Manure had been last applied to the experimental area in 1926, there had been no susceptible crop grown in the previous three years, and swedes or turnips had not been fed to stock on the area during the previous year, at least. There was no evidence of infection having been derived from any neighbouring crop. The only explanation

remaining is that the disease was contracted by the swedes from charlock (*Sinapis arvensis* L.), which was an abundant weed in the plots. When these plants were examined late in the season, lesions were present on the remains of the stems which yielded cultures of *Phoma Lingam* (strain II B). This fungus produced dry rot in swede roots in the laboratory, but its parasitic powers under field conditions have not yet been fully investigated. Buddin has recorded *Phoma Lingam* on the beaks of siliquas of *Brassica alba* (3), and it has since been suggested that this plays a large part in carrying over the disease (1). The facts recorded do not settle the question whether the swede crop contracted infection from the charlock or *vice versa,* but the former is more likely. The dry rot parasite could probably survive until the following season on dead charlock stems, as it has been proved here to do on dead cabbage stalks in the garden, or possibly on the seeds.

Experiment at Mountmellick, 1931. — Additional evidence regarding the relative importance of soil contamination and seed infection was secured in a special experiment conducted at Mountmellick in 1931, in co-operation with the Agricultural Instructor, Mr. Tynan. On account of complaints regarding the prevalence of dry rot in the district, seed belonging to four varieties was mycologically examined and divided into two parts, one of which was disinfected by Clayton's method, while the other was not. The two lots of seed were then sown under Mr. Tynan's direction on land which had carried a diseased crop in the previous year, and on clean land. The results are shown in the following table:—

TABLE 7.—Mountmellick Experiment, 1931, comparing contamination in soil and seed.

Variety.	No. of infected seeds.	P. C. dry rot by count.			
		Contaminated Soil.		Clean Soil.	
		Treated seed.	Untreated seed.	Treated seed.	Untreated seed.
Incomparable	3/2000	20·2	23·6	0·8	1·9
Magnificent	0/2000	24·0	17·3	0·9	1·0
Magnum Bonum ...	0/2000	19·2	23·6	4·3	3·8
Tipperary	0/2000	38·5	22·8	9·7	4·1

Only one variety, Incomparable, showed any infected seeds, and the number present (3 in 2,000) was large, as these figures go, yet there was no preponderance of dry rot in the resulting crop. It is again evident that if seed infection played any material part, which is improbable, its effect was extremely small in comparison with that of contamination persisting in the soil from the

previous year's crop. There are indications here also of the presence of undetermined sources of infection in the plots situated on clean soil, particularly in the case of the variety Tipperary.

The above conclusion as to the relative unimportance under most conditions of seed infection, in the proportions normally encountered, is borne out by a further test at Glasnevin in 1931. Seed of a variety supplied by Mr. Buddin, which showed 4 infected seeds in 5,000, was divided into two equal portions, one being disinfected by Clayton's method, and the other being untreated. Both lots were sown on adjacent plots of clean soil, which were dressed with artificial manures alone, and which were isolated by means of four drills of mangels. The results were as follows:—

Treated Seed ... 1·7 per cent. dry rot in resulting crop.
Untreated Seed ... 2·7 „ „ „ „

Here again seed infection of the proportions 4 : 5,000 produced only 2·7 per cent. of disease, while on the same farm, and under the same general conditions otherwise, contamination in the manure produced 18·9 per cent. of dry rot in that year.

Experiments in Clare, 1931.—Complaints were also received of severe outbreaks of dry rot of swedes in Clare, sometimes in cases in which no farmyard manure was used, and where an adequate rotation was said to be practised. Accordingly, it was decided to examine some of the seed of varieties which the local Instructor in Agriculture intended to sow in variety tests in different centres in the county. These tests were conducted without the use of farmyard manure. The results of the examination of the seed, and the final percentages of dry rot in the resulting crops at three centres, are shown in the following table. Thanks are due to Mr. W. Gahan, the Instructor, for taking these results and for the general conduct of the experiments:—

TABLE 8.—Results of sowing uniform seed of the same varieties at different centres in Clare (1931).

Variety.	No of infected seeds.	Per cent. dry rot by count at—		
		Gower (Sown May 1).	Churchtown (Sown May 8).	Curnanes (Sown June 10).
Knockadon	0/5000	21·0	15·2	not sown
Magnificent	1/5000	35·4	5·5	not sown
Majestic	0/5000	24·4	3·8	not sown
Tipperary	0/5000	26·7	not sown	5·6

It is evident that there was a high percentage of disease throughout at Gower, and that this was mainly due to local causes; for at Churchtown, which

is in the same district, and subject to the same general climatic conditions, there was considerably less infection. No clear connection between seed infection and dry rot in the crop can be traced.

In seeking for an explanation of the general prevalence of the disease at Gower, a factor is encountered which is of common occurrence on small farms. While at Churchtown the entire field was in lea for seven years before 1931, and no swedes or turnips were fed to stock on it, at least during 1929 and 1930, at Gower there had been two crops of swedes, in other portions of the field used, in 1929 and 1930. The latter crop suffered severely from dry rot, and some of the worst affected roots were left to decay in a heap by the fence. Thus, while technically a reasonable rotation was being followed here, its benefits, so far as disease control is concerned, were reduced owing to the proximity of the swede plots in successive years. In addition, some charlock was present in the field, while none was present at Churchtown.

Discussion of Field Experiments.—The most important question at issue in the foregoing experiments is the determination of the sources from which dry rot originates in the field. The results presented here, with the possible exception of those of 1928, emphasise the importance of contamination contained in the manure or soil, and minimise that of seed infection. The experiments were conducted during a sufficient period of years and under such a variety of conditions as to appear to warrant this general conclusion.

Very similar results have been obtained in other countries. A recent report of Reading University (1) states that soil is the primary source of infection in severe cases of dry rot. In Denmark (2) the disease is reported to originate from seed infection to some extent, but importance is also attached to infection originating from plant residues in manure and soil. Neill (15), in New Zealand, finds that seeds which have been treated with Semesan, and which appear free from dry rot in laboratory tests, produce crops which develop dry rot in the majority of cases in the field. Whitehead (22), in Wales, states that initial outbreaks are largely due to diseased material in the manure, but apparently considers that small amounts of seed infection may also be significant (23). In America, Clayton (5) finds, in the case of blackleg of cabbages and similar plants, that in addition to seed treatment a rotation of five or six years in the seed bed and three years in the field is necessary.

All the above evidence goes to show that sources of infection other than the seed are universally recognised as important. In view of this, and the evidence presented in this paper, there can be no doubt that Cunningham has overstated the case for seed infection in suggesting that if disease-free seed were available it would be possible to eliminate dry rot by following a rotation of one year in New Zealand.

The occurrence and significance of *Phoma Lingam* on other Crucifers, such as charlock (*Sinapis arvensis*), has yet to be examined thoroughly, as many weeds closely allied to swedes are common on cultivated land. In the experiments carried out in 1931 charlock occurred in the plots at two centres, and a serious

attack of dry rot was associated with it in both cases. In one of these experiments there were, in the case of certain plots, no known sources of infection either in the seed, manure, or soil, and suspicion was, therefore, thrown on the charlock when *Phoma Lingam* was found to occur on it late in the season. As already stated, Buddin (3) has recorded the fungus on *Brassica alba*. If it is found to occur generally, and to hibernate on cruciferous weeds, it will go far to account for some at present inexplicable outbreaks.

Dry Rot in Storage.

The attack of dry rot does not cease when swede roots are harvested and placed in storage in November or December for gradual use in the succeeding months. Superficial lesions, which are characteristic of the storage period, frequently develop in very large numbers all over the roots while they are in the pits. Many of these roots are usable, so that the percentage of infected specimens is no measure of the economic loss, but on the other hand soft bacterial rots not infrequently ensue, for the inception of which the presence of dry rot must in many cases be held primarily responsible.

From about the time the roots are pulled onwards, dry rot becomes much more common than previously, so much so that it is practically impossible to find a crop in which it does not develop subsequently. Thus, in November, 1929, a quantity of apparently healthy roots was saved from a crop showing very little dry rot, and set aside in order to allow any incipient infections to develop. By January 9, 1930, 16 per cent. of the roots were found to be diseased. On the latter date the remaining apparently healthy roots were stored in two small pits, to one of which were added five marked roots badly attacked by dry rot. The result, as determined on April 11, was as follows:—

Pit to which diseased roots were added—68 per cent. dry rot.

Control pit—66 per cent. dry rot.

This result is to be interpreted, not as indicating that the disease cannot and does not spread in the pit, but that the sources of infection, in the form of incipient lesions or superficial contamination, were about equally abundant in both the pits. The addition of the five badly diseased roots to one lot was, under the circumstances, without any marked effect, but the disease uniformly reached its highest proportions (77 per cent. on the average) in their immediate vicinity.

Another attempt was made in the following year to compare the keeping quality of the roots from a diseased and from a comparatively healthy crop, both grown at Glasnevin. The former had contained 19 per cent., and the latter 1 per cent., of dry rot in the field, and apparently healthy roots from

both were pitted separately under the same conditions on November 24, 1931. The pits were opened on March 24, 1932, with the following results:—

	Total roots.	Per cent. dry rot.	Per cent. usable.
Diseased crop ...	1,153	100	57
Healthy crop ...	1,296	78	94

As in the previous experiment, there was a remarkable spread of dry rot in both lots of roots, even when only 1 per cent. of infection had occurred in the growing crop. In this case, however, 94 per cent. of the total crop was still fit for use near the end of March, while when the disease had been more abundant in the field, 43 per cent. of the apparently healthy crop which was put into storage rotted away before that time. The appearance presented by the two lots when the pits were opened was strikingly different.

The remarkable extent to which dry rot develops in roots after storage is attributable to a number of factors. It has already been shown that non-growing roots have less resistance than growing ones. The humid atmosphere of the pits would also be favourable for the development of the disease. Most of the infection which develops in storage is attributed to the repeated handling of the roots in the operations of pulling, loading, unloading, and building them into heaps, which are usually conducted in weather favourable for spore exudation. It is also possible, in cases in which the disease is abundant on the leaves, that many of the roots may become contaminated from this source when being pulled. In this connection, it is commonly held that the crop keeps better if it is allowed to stand in the field without being pulled, than in pits. The foregoing experiments appear to indicate that the greater part of the dry rot found when pits are opened originates from superficial contamination, or incipient lesions, already present on the roots as they come from the field. At the same time there is evidence in both experiments of a further spread of the disease in the pits. Spore exudation has been observed in storage, and the pit covering is usually sufficiently open to allow rain to trickle in, and so spread the disease. The general conclusions reached by Whitehead and Jones (22) are somewhat similar.

Relative Susceptibility of Varieties to Dry Rot.

The possibility that the fleshy roots of the variety Caledonian may be particularly susceptible to dry rot, and that this may explain the excessive amount of disease which was present in this variety in the field experiments in 1928 (14), has already been discussed. Subsequent work on this question has, on the whole, failed to confirm the presence of outstanding susceptibility in this or any other variety of swede. Observations in the field have indicated that fluctuation of the other factors affecting the incidence of dry rot obscures any

differences in susceptibility which may exist. This indicates that the latter are probably not of great magnitude. A somewhat similar conclusion had already been drawn from reports furnished by the Agricultural Instructors (14).

A certain number of laboratory trials was also made in 1928 and 1929 with six selected swede varieties, but the variations in the results were so considerable that no certain conclusion is possible. There were indications that the varieties Up-to-Date and Incomparable showed some measure of resistance, while Tipperary, Best-of-All, Magnificent, and Caledonian were more susceptible. No immune varieties of swede are known, and turnips as a class have been observed to be less susceptible than swedes.

Summary.

The symptoms and development of dry rot on swede seedlings, leaves, fleshy roots, siliquas, and seeds are described.

Dry rot of swedes and turnips is caused by *Phoma Lingam*. A number of fungi occur on diseased swede plants which are considered forms of this parasite, but the one which is exclusively associated with typical dry rot of the roots in Ireland, and is predominant on other organs, is identical with the American cabbage black-leg organism, and with strain II A of *Phoma Lingam* from New Zealand. A table is given showing the number of times each form was isolated from the various organs of diseased swede and other plants (p. 500).

On other plants than swede (and possibly turnip), a different form of *Phoma Lingam* (II B) predominated, producing a disease less severe than the American black-leg. The practical absence of the latter from Ireland is attributed to prevailing environmental conditions.

Strain II A consistently produced dry rot in swede roots in the field and in the laboratory. Strain II B, with one exception, failed to do so in the field, while producing an indistinguishable rot in non-growing roots in the laboratory.

In Ireland there is a close approach to the conditions that strain II A is adapted to on active parasitic life on swedes (and possibly turnips), and strain II B on cabbages and related plants.

Various weakly parasitic or saprophytic forms of *Phoma* corresponding for the most part with Cunningham's strains I A and III, and a somewhat more strongly parasitic form corresponding to I B, are provisionally retained in the species *Phoma Lingam*, though some show affinity to other described species of *Phoma*.

Tests of the parasitism on swede root of strains of *Phoma Lingam* should be made on growing plants.

Phoma Lingam is present in the outer portion of the testas of infected seeds, but does not occur in the embryos of viable seeds.

Dry rot may originate from at least three sources, namely—(1) the seed;

(2) diseased roots of the previous year which survive in the soil; (3) similar roots present in farmyard manure.

A detailed description is given of the progress of dry rot originating from each of these sources, and the relative importance of the latter is discussed.

A total of 164,050 seeds belonging to 42 commercial lots of swede and turnip seed was examined, and 10 lots were found to be infected. The number of infected seeds was very small in every case, averaging 1 in 6,310 seeds.

Phoma Lingam was found to grow in sterilized potting soil, but not in unsterilized field soil.

Dry rot develops extensively in storage pits, and the sources of infection are discussed.

No well marked resistance to dry rot was found to occur in swedes.

Extensive field experiments, extending over a period of three years, showed that the amounts of infection usually present on the seed were not capable of producing outbreaks of dry rot as serious as those originating from contamination in soil or manure. In certain experiments, charlock (*Sinapis arvensis*) occurred in association with severe attacks of dry rot, and the presence of *Phoma Lingam* on these plants was discovered.

LITERATURE CITED.

1. ANON.—Reports on the work of Agricultural Research Institutes, and on certain other Agricultural Investigations in the United Kingdom, 1929–1930.
2. ANON.—Afsvampning af Roefro. Stat. Forsogs. i Plantekultur, 188 Med., 1932.
3. BUDDIN, W.—Min. of Agric. and Fish. Monthly Summ. of Plant Pests, etc., Report for November, 1930.
4. CARRUTHERS, W.—Annual Report for 1903 of the Consulting Botanist. Jour. Roy. Agric. Soc., England, **64**, 296–309, 1903.
5. CLAYTON, E. E.—Black-leg disease of Brussels sprouts, cabbages and cauliflowers. N.Y. State Agric. Exp. Sta., Geneva, Bul. **550**, 1927.
6. CLAYTON, E. E.—Seed treatment for black-leg disease of crucifers. N.Y. State Agric. Exp. Sta., Geneva, Tech. Bul. **137**, 1928.
7. CUNNINGHAM, G. H.—Dry rot of swedes and turnips: its cause and control. New Zealand Dept. of Agric., Bul. **133**, 1927.
8. GRIMES, M., O'CONNOR, M., and CUMMINS, H. A.—A study of some *Phoma* species. Trans. Brit. Myc. Soc., **17**, 97–111, 1932.
9. HENDERSON, M. P.—The black-leg disease of cabbage caused by *Phoma Lingam* (Tode). Desmaz. Phytopath. **8**, 379–431, 1918.
10. HOLMES-SMITH, E.—Min. Agric. and Fish., Monthly Sum. of Plant Pests, etc. Report for November, 1930.

11. Johnson, T.—Some root crop diseases in Ireland. Jour. Dept. of Agric. and Tech. Instr., Ireland, **4**, 258–263, 1903.
12. Lund, S., og Hjalmar, K.—Morfologisk-anatomisk Beskrivelse af *Brassica oleracea* L., *B. campestris* L., og *B. napus* L. (Havekaal, Rybs og Raps.) samt Redegjorelse for Bestoonings og Drykningsforsog med disse Arter. Bot. Tidsskrift (Kjobenhavn), **15**, 1–150, 1886.
13. Murphy, P. A.—The connexion between dry-rot of swedes in New Zealand and British seed. *Nature*, July 7, 1928.
14. Murphy, P. A., and Hughes, W.—The dry-rot of swedes and turnips caused by *Phoma Lingam* (Tode) Desm. Jour. of Dept. of Agric., Irish Free State, **29**, 29–40, 1929.
15. Neill, J. C.—Dry-rot of swedes: some field observations and experiments on control. New Zealand Jour. of Agric., **39**, 86–93, 1929.
16. Neill, J. C., and Brien, R. M.—A method to obtain dry rot infected seed. *Ibid.*, **42**, 433, 1931.
17. Nielsen, O.—Undersogelser over "black-leg" paa Kaal og Torforraadnelse paa Kaalroer. Tidssk. f. Planteavl, **38**, 131–154, 1932.
18. Orton, C. R., and Rex, E. G.—Botany and Plant Pathology. Thirty-seventh Ann. Rep. Pennsylvania Agric. Exp. Sta., 1924.
19. Potter, M. C.—A new *Phoma* disease of the swede. Jour. Board of Agric., England, **6**, 448–456, 1900.
20. Rostrup, E.—Phoma-Angriff bei Wurzelgewachsen. Zeitschr. f. Pflanzenkrank., **4**, 322–323, 1894.
21. Walker, J. C.—The hot water treatment of cabbage seed. Phytopath., **13**, 251–253, 1923.
22. Whitehead, T., and Pritchard Jones, W. A.—"Dry-rot" of swedes. Welsh Jour. of Agric., **5**, 159–175, 1929.
23. Whitehead, T.—Dry-rot of swedes. Second progress report. Welsh Jour. of Agric., **6**, 289–295, 1930.

Description of Plates.

Plate 46.

Fig. 1.—Cotyledon from infected swede seed, showing primary stage of dry rot from this source, 15 days after sowing. (× 3/2).

Fig. 2.—Dry rot lesion on swede leaf showing perforation in the centre and irregular arrangement of the pycnidia. (× 2).

Fig. 3.—Pycnidia of *Phoma Lingam* occurring abundantly on a wilted swede leaf. (× 2).

Fig. 4.—Swede seedling from infected seed being killed by the dry rot organism. One cotyledon was destroyed before it emerged from the seed coat, and the parasite grew into the hypocotyl. Slightly enlarged.

Fig. 5.—Swede siliquas infected with dry rot, showing abundant pycnidia, and the malformation which sometimes occurs. Slightly reduced.

Fig. 6.—Swede seeds from infected siliquas plated on potato glucose agar, showing the dry rot organism growing from seed, three days after plating. Slightly reduced.

Figs. 7, 8, 9.—Stages in the development of dry rot on swede roots. (× 1/4).

PLATE 47.

Fig. 10.—Lesions produced by laboratory inoculations with mycelium of *Phoma* belonging to strain I A. Slightly reduced.

Fig. 11.—Type of lesion produced in laboratory inoculation with *Phoma* belonging to strain II B. Fungi belonging to strain II A and the actively parasitic members of I B produce a similar lesion in the laboratory. (× 5/9).

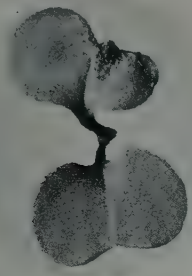
Fig. 12.—Typical dry rot lesion produced in field inoculation by *Phoma Lingam* strain II A. (× 1/3).

Fig. 13.—Natural lesion on a swede root from which a *Phoma* of strain II B was isolated. This is not typical dry rot. Nat. size.

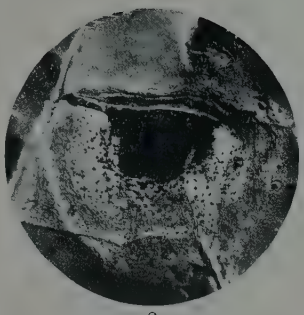
Fig. 14.—Culture of *Phoma Lingam,* strain II A, on potato glucose agar showing lobing, after 16 days' growth. Nat. size.

Fig. 15.—Culture of *Phoma* strain I A on potato glucose agar showing colony after 6 days' growth. Slightly reduced.

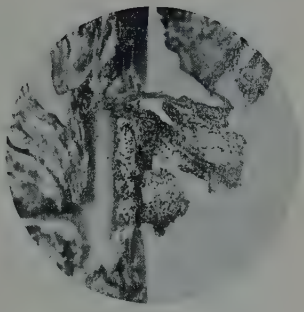
Fig. 16.—Culture of *Phoma* belonging to strain II B, on potato glucose agar, after 12 days' growth. Slightly reduced.



1

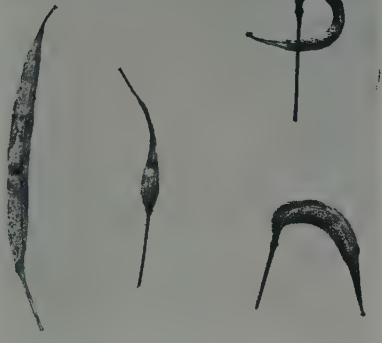


2

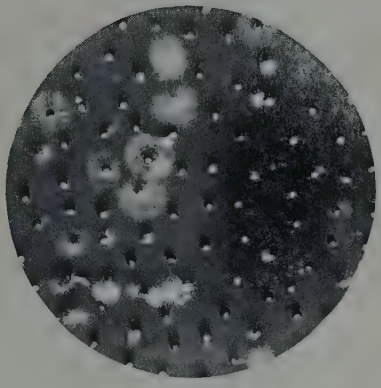


3

4



5



6

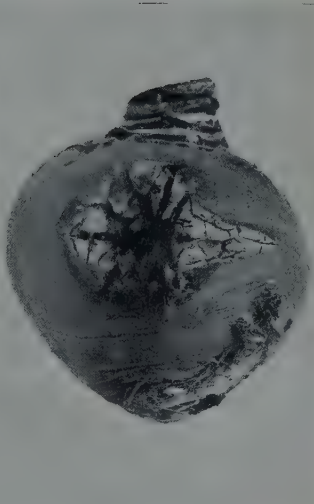
7

8

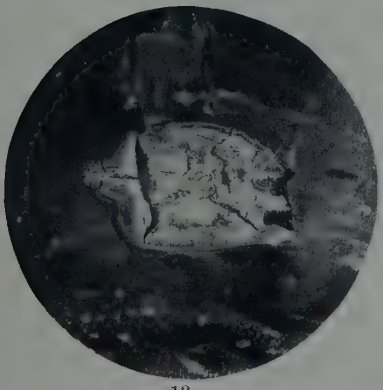
9

10

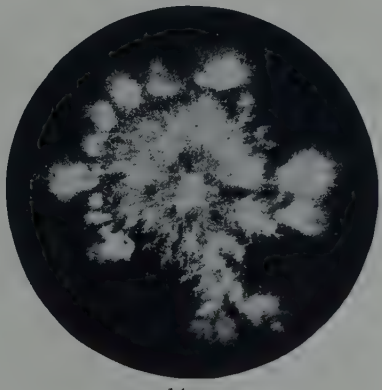
11



12

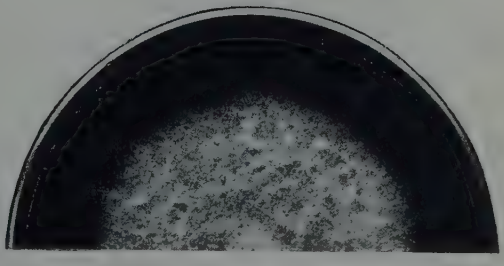


13



14

15



16

No. 35.

A CLASSIFICATION OF THE BIOLOGICAL ELEMENTS, WITH A NOTE ON THE BIOCHEMISTRY OF BERYLLIUM.

By WILLIAM ROBERT FEARON, M.B., Sc.D., F.T.C.D., F.I.C.

[Read December 20, 1932. Published separately February 20, 1933.]

A Classification of the Biological Elements.

Of the ninety-two elements believed to constitute the universe, at least thirty-six have been found to be associated in one way or another with the fabric of life. From them are derived all the compounds and systems of biology, and, whether the organism be regarded as an episode in the history of matter or of spirit, its behaviour is restricted by the chemical properties of its constituents. Biological elements may be grouped (*a*) *chemically*, as metals and non-metals; (*b*) *physiologically*, as essential and non-essential elements; and, (*c*) *biologically*, as plant and animal constituents.

While these and similar classifications are useful for particular ends, it is simpler at the outset to take the elements in group order according to the periodic table, making a sub-division into *variable* and *invariable* elements, and recognising a quantitative distinction between *primary* elements, *secondary* elements, and *micro-constituents*.

1. *Invariable Primary Elements.*—**Hydrogen, Carbon, Nitrogen, Oxygen, Phosphorus.**—These make up the greater part of the organism, representing, individually, from 1 to 60 per cent. of the total weight. They are found in all known forms of life, and determine largely its physical structure.

2. *Invariable Secondary Elements.* — **Calcium, Magnesium, Sodium, Potassium, Iron, Sulphur, Chlorine.** — These are equally necessary for life, but occur in much smaller quantities, usually from 0·05 to 1 per cent. of the total weight of the organism.

Among the vertebrates, calcium is a primary element.

In many plants, sodium, calcium, and iron may be mere micro-constituents.

3. *Variable Secondary Elements.*—**Zinc, Titanium, Vanadium, Bromine.**—These elements reach a relatively high concentration in certain species where they are obviously necessary for life, but their occurrence and importance in other species are often doubtful, and they are absent from many organisms.

4. *Invariable Micro-constituents.*—**Copper, Boron, Silicon, Manganese, Fluorine, Iodine.**—These are believed to be present in all forms of life. The concentrations are minute; usually less than 0·005 per cent. of tissue weight.

Silicon may appear as a secondary element in certain plants and in marine organisms.

5. *Variable Micro-constituents.*—**Lithium, Rubidium, Caesium, Silver, Beryllium, Strontium, Cadmium, Germanium, Tin, Lead, Arsenic, Chromium, Cobalt, Nickel.**—Some of these have only been detected in a few species, and their functions are quite obscure.

6. *Contaminants.*—**Argon, Barium, Mercury, Thallium, Selenium, and Gold.**—An additional seven elements have been reported as occurring in isolated instances in particular individuals.

There appears no reason to regard them as other than fortuitous in origin and negligible in function.

Group 0	Group I a	Group I b	Group II a	Group II b	Group III a	Group III b	Group IV a	Group IV b	Group V a	Group V b	Group VI a	Group VI b	Group VII a	Group VII b	Group VIII
	H 1.008														
He 4.00	Li 6.94		Be 9.02		B 10.82		C 12.00		N 14.01		O 16.00		F 19.00		
Ne 20.2	Na 22.99		Mg 24.32		Al 26.97		Si 28.06		P 31.03		S 32.06		Cl 35.46		
A 39.91	K 39.09	Cu 63.37	Ca 40.07	Zn 65.38	Sc 28.06	Ga 69.72	Ti 48.1	Ge 72.60	V 50.96	As 74.96	Cr 52.01	Se 79.2	Mn 54.93	Br 79.92	Fe 55.84 Co 58.94 Ni 58.69
Kr 82.9	Rb 85.44	Ag 107.88	Sr 87.63	Cd 112.4	Y 88.9	In 114.8	Zr 91	Sn 118.7	Cb 93.1	Sb 121.7	Mo 96	Te 127.5	Ma	I 126.9	Ru 101.7 Rh 102.9 Pd 106.7
Xe 130.2	Cs 132.8	Au 197.2	Ba 137.4	Hg 200.6	La * 138.9	Tl 204.4	Hf 178	Pb 207.2	Ta 181.5	Bi 209.0	W 184.0	Po 210	Re 187	–	Os 190.8 Ir 193.1 Pt 195.2
Nt 222.4	–		Ra 226		Ac 228		Th 232		–		U 238.2				

* And rare earths.

Position of the Biological Elements in the Periodic Table.

When the biological elements are marked off in the periodic table it will be seen that a line can be drawn separating them from the non-biological elements. All elements above this line have been detected in one or more organisms. No element below the line has been shown to be an essential or a predictable constituent of any organism.

Beryllium as a Biological Element.

During the tabulation of the elements it was noticed that only one gap occurred above the dividing line, there being no available evidence that

beryllium had been detected in any organism. A search through the literature failed to afford any indication that this particular metal had ever been sought It is not included in any of the spectrographic surveys of milk, or of animal tissues and secretions (Ramage, *et al.*, 1929, 1931; Dutoit and Zbinden, 1929). In view of these negative results, a search was made in plant material, especially from regions relatively rich in the element. This work was begun with the help of Mr. J. A. Wallace, but has not been pursued beyond the examination of a few species. The results obtained, however, show that beryllium is a frequent but not an essential micro-constituent of the lichens *Parmelia saxatilis* and *Xanthoria parietina.*

Detection of beryllium in plant ash.—The analytical plan adopted was on the lines indicated by Ainsworth Mitchell (1932) and Booth and Frary (1932). The beryllium was precipitated in the aluminium fraction, and separated by several different methods, according to the recommendations of Booth and Frary. The metal was identified (1) by formation of the characteristic double salt with uranyl acetate (Caglioti, 1927), and (2) by the formation of a red "lake" on addition of curcumin (Kolthoff). The latter reaction is very sensitive, and will detect at least 1 mg. Be per litre, in the absence of Fe and Al. The preparation of the material was somewhat troublesome as the plants are usually contaminated with soil particles that resist removal. Specimens were collected from exposed rocks, and the cleanest parts of each thallus were selected.

After prolonged, continuous, and intermittent washing, the material was incinerated and extracted. The samples of ash obtained ranged from about 5 to 25 g., representing 4–5 per cent. fresh tissue.

The amount of beryllium varied greatly in different batches, and probably depends on local conditions. Specimens of *Xanthoria* found growing on the exposed granite of Kingstown pier contained no detectable traces of the metal, and these negative findings were confirmed by a spectrographic examination of the ash undertaken by Messrs. Adam Hilger of London.

The only qualitative analysis of *Parmelia* ash that we have been able to find is that of Keegan (1916), who reports that 5·5 per cent. of the total ash is composed of "silica and earthy matter." Beryllium is not a rare element, and it is likely that beryllia has often been mistaken for alumina in many biological analyses.

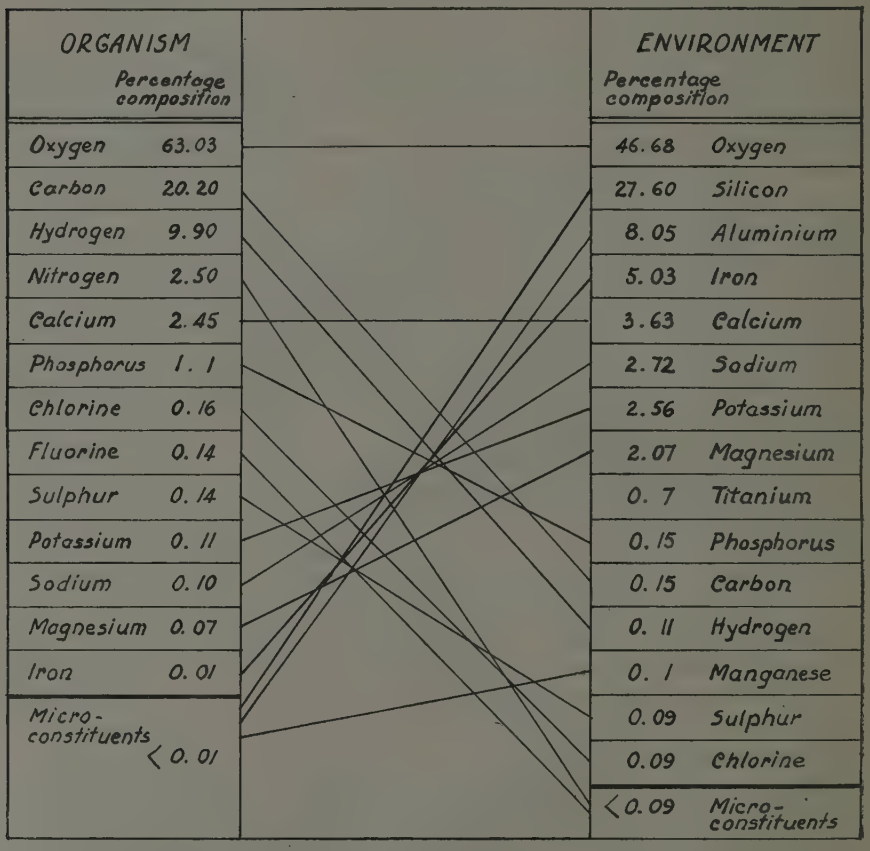
The biochemistry of beryllium.—The metal has attracted little notice. Moore (1921) claims that, like uranium, it can catalyse the photosynthesis of carbonic acid in solutions exposed to ultra-violet radiation. Branion, Guyatt, and Kay (1931) describe a form of experimental rickets produced by substituting beryllium carbonate for calcium carbonate in the standard dietary of the rat. Administration of vitamin D is unable to prevent or cure this condition.

Significance of the Biological Elements.

While many unrecognised factors may be concerned in the selection and assembling of the elements that constitute the living organism, two, at least,

are sufficiently distinct to demand consideration. These are suitability and availability.

The suitability of the biological elements would appear to be set beyond discussion by the mere fact of their presence in the organism. Underlying this, however, is the deeper question as to whether the determinants of organisation are, in turn, determined by the properties of the elements, or whether there is a process of chemical selection at work gradually altering and improving the framework of life.



Relative Composition of the Organism and the Environment.

L. J. Henderson has developed the concept of environmental fitness, and has shown that the unique properties of the primary elements, carbon, hydrogen, and oxygen, endow their compounds with the maximal fitness for the manifestation of life. This concept has not yet been extended to the other elements, although it is interesting to speculate on the biological importance of magnesium

and iron. Without the former there would be no chlorophyll, without the latter there would be no hæmoglobin.

The availability of the biological elements fixes the limits to the expansion of life. This was recognised by Liebig, in 1843, and expressed as his familiar "law of the minimum."—The yield of any crop is determined by the nutritive constituent present in least amount.

When the percentage compositions are compared it is seen that a marked difference exists between the composition of the organism and its terrestrial environment. Thus, silicon and aluminium, the elements that make up one-third of the Earth's crust, only appear as micro-constituents of man; whereas hydrogen, carbon, and nitrogen, the building stones of life, are only minor constituents of the environment. Rough data such as these show the power of the living organism to select the necessary biological elements irrespective of their relative concentrations, and its inability or reluctance to utilise some of those most readily available.

Summary.

The chemical elements of living organisms may be classified as:—(1) invariable primary elements, (2) invariable secondary elements, (3) variable secondary elements, (4) invariable micro-constituents, (5) variable micro-constituents, and (6) contaminants.

The periodic table can be divided into two sections, the upper one containing all the thirty-four biological elements, and the lower one containing only non-biological elements.

Beryllium has been detected as an occasional constituent of the lichens *Parmelia saxatilis* and *Xanthoria parietina*.

REFERENCES.

1. BOOTH, H. S., and S. G. FRARY (1932), J. Phys. Chem., **36**, 2640.
2. BRANION, E. D., B. L. GUYATT, and H. D. KAY (1931), J. Biol. Chem., **92**, xi.
3. CAGLIOTI, V. (1927), J. Chem. Soc. Abs., **104**, 1046.
4. DUTOIT, P., and C. ZBINDEN (1929), C. R. Acad. Sci., **188**, 1628; **189**, 172.
5. FOX, H. M., and H. RAMAGE (1931), Proc. Roy. Soc., B., **108**, 157.
6. KEEGAN, P. (1916), Chem. News, **114**, 74.
7. KOLTHOFF, I. M. (1928), J. Amer. Chem. Soc., **50**, 373.
8. LOTKA, A. J. (1925), "Elements of Physical Biology."
9. MITCHELL, C. A. (1932), "Recent Advances in Analytical Chemistry," Vol. 2.
10. MOORE, B. (1921), "Biochemistry."
11. RAMAGE, H. (1929), Nature, **123**, 601; (1930), **126**, 279.
12. SHELDON, J. H. and H. RAMAGE (1931), Biochem. J., **25**, 1608.

DEPARTMENT OF PHYSIOLOGY,
TRINITY COLLEGE, DUBLIN.

No. 36.

EXPERIMENTS ON THE SUITABILITY OF SOME RECTIFIER PHOTO CELLS FOR THE MEASUREMENT OF DAYLIGHT.

By H. H. POOLE, Sc.D.,

AND

W. R. G. ATKINS, Sc.D., F.R.S., F.Inst.P.

[Read February 28. Published separately April 20, 1933.]

Photo cells of the dry rectifier type introduced by Lange (7) possess obvious advantages for the measurement of daylight in the field and under water. Before using them for such purposes we have, accordingly, carried out some laboratory tests on a number of these cells to ascertain (*a*) the relation between the illumination and the resulting current up to intense illuminations, (*b*) the effect of the resistance of the galvanometer on this relation, (*c*) the effect of rise of temperature of the cells due to exposure to bright light, (*d*) the colour sensitivities of various types of cell, and (*e*) the effect of time and continuous exposure to bright sunlight on the sensitivity.

Varieties of Cells examined.

Tests have been carried out on the following cells:—

A, a cell made by M. Auger (4), who very kindly gave it to one of us. It is of the cuprous oxide type, 2 cm. in diameter, the front conducting film being, we understand, of sputtered gold, and the window of thin glass. We modified the vulcanite mounting of the cell so as to adapt it to our special purpose by decreasing the screening of low-angle light. We also removed some of the vulcanite from the back of the case so as to facilitate the escape of heat from the copper back. Unfortunately we found on reassembling the cell that the sensitivity for direct light had been somewhat reduced, possibly owing to some slight damage to the gold film. The results plotted in Figs. 2 and 4 were obtained after this change had been made.

Sx 80 and *Sx 81*, two "Serpidox" commercial cells of the Auger type, but mounted somewhat differently, diameter 1·7 cm., made by the Soc. de Recherches et de Perfectionnements Industriels, Puteaux (Seine). We were able to modify the vulcanite mounting of these cells so as to reduce the screening of low-angle light and to facilitate the escape of heat without interfering with the actual cells, so that their sensitivities for direct light were unchanged. The sensitivities of these two cells for all illuminations were almost identical when new.

S, supplied by Messrs. Siemens and Halske, a somewhat similar cell, 1·7 cm. in diameter, mounted under a Cellon (acetyl cellulose) window in a very compact metal case, which gives very little screening of low-angle light, and is well adapted for the dissipation of heat. The conducting film is of silver.

All the above cells are of the cuprous oxide type, with gold or silver films, and inspection of Fig. 4 will show that their colour sensitivities are very similar.

We have also tested two other cells, which have entirely different characters. *Ws*, a Westinghouse red-sensitive cell, diameter 2·5 cm., in a moulded insulation case under glass, with a moderately good exposure to oblique light, but poor heat dissipation. This is like the original Lange cell in colour sensitivity, and presumably the film in front is of copper.

Wn, the selenium rectifier cell of Bergmann (5), manufactured by the Süddeutschen Apparate-Fabrik, Nürnberg, and now under license by the Weston Electrical Co., from which we obtained our cell. In this a layer of selenium is melted on to an iron base, and a film of noble metal is subsequently deposited by sputtering. Its diameter is 4 cm., and it is mounted in a moulded insulation case similar to that of *Ws* under glass. Having a much larger effective area, this cell is much more sensitive than any of the others when used with a low resistance.

The Relation between Light Intensity and Current.

Preliminary tests showed that the current light relation was rarely linear, and that its form depended greatly on the resistance of the galvanometer used. The effect of rise of temperature was complex, increasing the sensitivities in weak light and decreasing them in strong light, and depending on the galvanometer resistance. A low resistance galvanometer gave a better approach to a

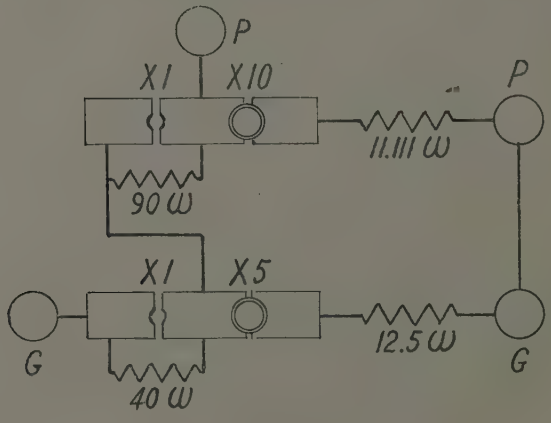


FIGURE 1.

linear relation and a smaller temperature effect. A 10-ohm pointer galvanometer of the Cambridge "Versatile" type was accordingly adopted as a standard. This instrument is convenient for field measurements, as it is fairly light (1·8 kg.), and does not need very accurate levelling. One scale division equals 2 μa, the maximum reading being 240 μa. For larger currents a shunt such as shown in Fig. 1 may be used. This was made up to our specification by Messrs. Tinsley, and is so constructed that by suitable arrangement of the two plugs ratios 1 : 1, 5 : 1, 10 : 1, and 50 : 1 may be obtained, the total resistance of the shunt with 10-ohm galvanometer remaining at 10 ohms throughout. This shunt was not available for the tests described here, so a "Post Office" resistance box was used when required to form an 11 : 1 shunt, 100 ohms being in series with the galvanometer and 11 ohms in parallel with the 110 ohms so formed. The combined resistance of this arrangement is also 10 ohms. With *Wn* in brighter light the series resistance was increased to 1000 ohms, giving a ratio 92·8 : 1, but increasing the effective resistance to 10·9 ohms. Additional resistances of 100 or 1000 ohms were introduced as required into the cell circuit, so that their effects might be studied.

Each cell was mounted in turn on a photometer bench, so that its distance from a 220 volt 250 watt projector type gas-filled lamp could be quickly read off. The lamp was mounted in a lantern, to cut off unwanted light, with its filament occupying a vertical plane area, about 1 cm. square, perpendicular to the axis of the bench. The exact position to be ascribed to the filament constituted the chief uncertainty in the required distance, but this probably did not exceed 0·5 mm., which would cause an error of about 1·5 per cent. in the estimated illumination at the shortest distance used (6·6 cm.). Allowance had to be made for the optical effect of the water cell, when it was used to reduce the heating, on the effective distance, which it reduced by 6 mm., and for the finite sizes of the source and of the cell, which decreased the illumination, as found by the inverse square law, by about 6 per cent. for the largest cell at the shortest distance.

The voltage at the lamp terminals was maintained as constant as possible by hand regulation at 210. The candle-power of the lamp at this voltage was compared with that of an open carbon arc used as we have already described (8) for standardising photo-electric cells. We have hitherto used the arc for this purpose, as being the only available source even remotely resembling daylight in spectral distribution, and as being approximately reproducible at any time. Three cells *A*, *S*, and *Wn* were used for this comparison, and gave, as mean values for the candle-power of the projector lamp, 334, 349, and 383, respectively. The differences are to be expected, in view of the different colour sensitivities of the three cells. Since, for our present purpose, an accurate knowledge of the absolute illumination is not required, we may take the value 383 for the candle-power without serious error, the lower values obtained with the other two cells being attributable to their insensitivity to red light. The water cell was found to transmit 91 per cent. of the light.

Fig. 2 shows how the current varies with the illumination for the various cells with external resistances 10 ohms and 1010 ohms, the curves for the larger resistances being marked with an asterisk. Owing to the large size, and hence the great sensitivity, of *Wn* its readings have been divided by 5 in plotting its 10-ohm curve. *Ws*, on the other hand, being only sensitive to red light, gave very small readings, which have been multiplied by 5 in plotting both its

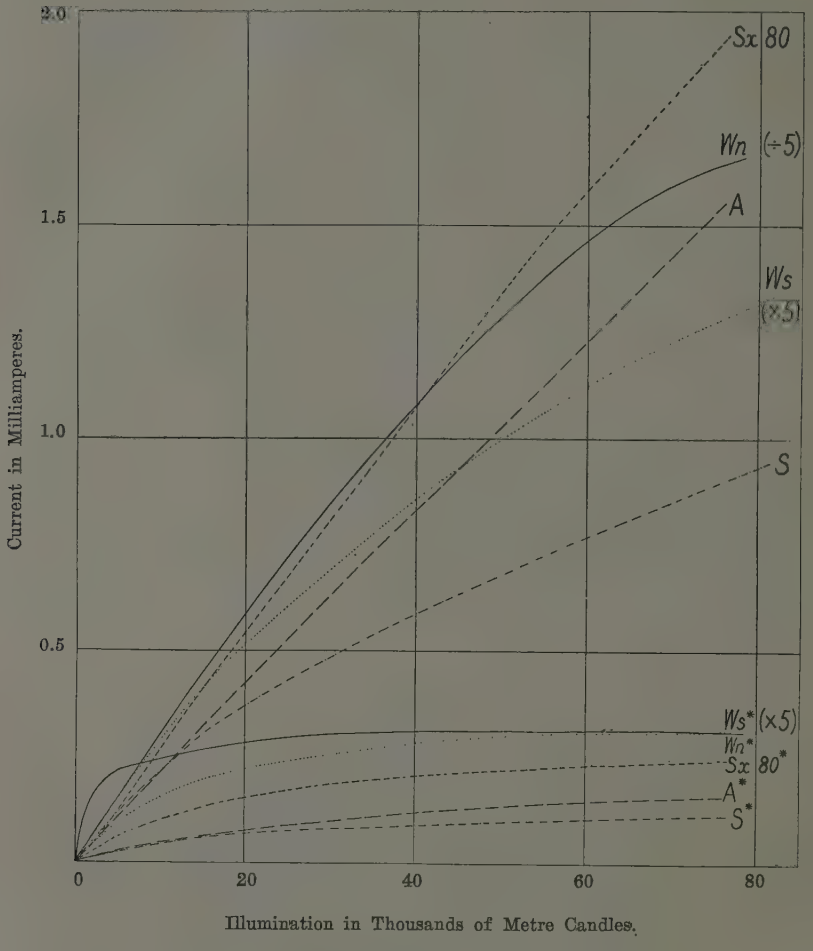


FIGURE 2.

curves. Each of the curves represents the results of 8 or 10 readings, made as quickly as possible in ascending order, the water cell being inserted to reduce

the heating effect. During a similar test with *Sx 81* a thermometer touching the copper back showed a rise less than 3° C.

It will be seen that the cell *A* made by M. Auger gives (with a 10-ohm galvanometer) a current much more nearly proportional to the light than any of the others. The Serpidox cells are exceptional in showing a considerably reduced sensitivity in weak light. The scale of the figure is too small to bring out this effect very prominently, but it would be of importance in the measurement of small daylight factors. With most of the cells the sensitivity falls for strong light, the effect becoming so large with a high resistance galvanometer as to prohibit its use for the measurement of intense light. The range covered rather exceeds that to which the cells would generally be exposed in the measurement of daylight, since for this they should be exposed behind an opal glass, so as to enable them to record oblique light with fair accuracy, while the bench tests were carried out on bare cells. The effect of this glass placed immediately in front of a cell in reducing the reading varies according to the construction of the cell case. Thus with *S,* which has an excellent exposure to low-angle light, the transmission of the opal glass for a normally incident beam is 56 per cent., while with *Wn,* in which a good deal of the low-angle light scattered by the diffusing layer is cut off, the figure is only 42 per cent. In all cases, however, an opal glass would reduce the intensity of the brightest sunlight to within the range covered.

Curves showing the current, and also the open circuit P.D., given by *Sx 81* in daylight of varying intensity, as determined with a vacuum potassium cell, have been given by us elsewhere (3).

The Effect of Alteration in Temperature.

Fig. 3 illustrates the effect of rise of temperature on the readings of cells *Sx 81* and *Wn*. Accurate measurements of the temperature effect are not easy, owing to the difficulty of either controlling or measuring the temperature of the active layer when exposed to strong light. By removing the water cell, however, and taking readings first in ascending, and immediately afterwards in descending, order, the effect of the temperature rise on the sensitivity for a given galvanometer resistance is made evident. Owing to the absence of the water cell, and to the fact that almost twice as many readings were taken in ascending order as in Fig. 2 (and repeated in descending order), the rise in temperature was far larger. The readings of a thermometer inserted in a hole in the vulcanite case of *Sx 81* touching the copper back are plotted in the figure as well as the ascending and descending branches of the current illumination curves for the two cells for galvanometer resistances 10, 110, and 1010 ohms. As these tests on *Sx 81* were carried out before the vulcanite back had been opened, the rise in temperature of *Wn* was probably somewhat similar, it also having an enclosed back. The illumination was not raised to the same

extent with *Wn*, but, owing to its larger absorbing area, it probably got at least as hot.

Comparison of Figs. 2 and 3 shows the depressing effect of heat on the sensitivity of *Wn* for very bright light. This is more clearly brought out for

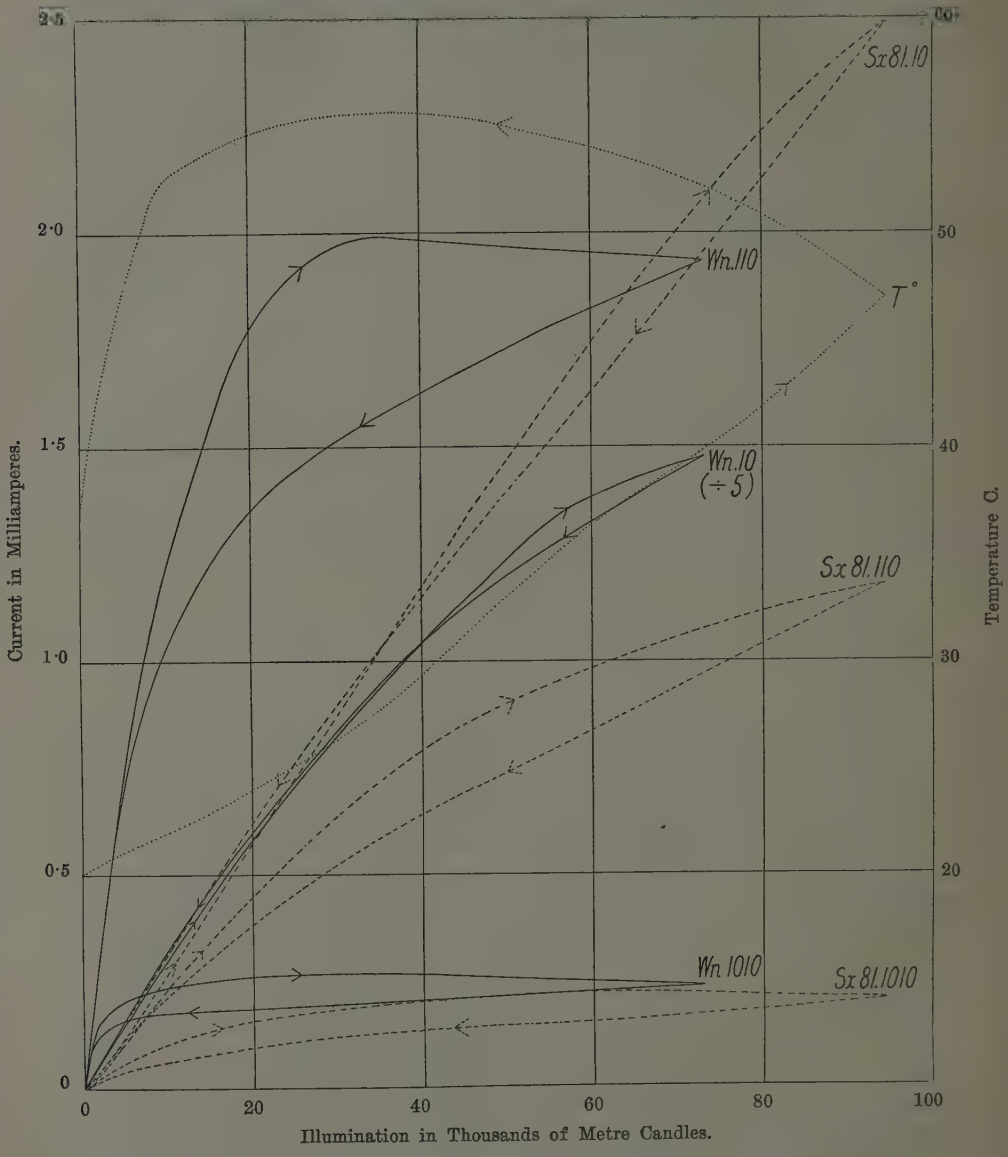


FIGURE 3.

both cells by comparing the ascending and descending branches in Fig. 3, the latter representing readings at higher temperatures. Above about 40,000 metre candles both cells read lower when hot, even with a galvanometer resistance of 10 ohms. When used to measure daylight, however, with an opal and probably also a water cell in position, the illumination falling on the photo-cell would rarely exceed 40,000 metre candles, and for all lower values heat has the effect of increasing the sensitivity, slightly in the case of *Wn*, and considerably in that of *Sx*. Experience with *Sx 81* in the field has confirmed this rise of sensitivity.

If the cells are used with high resistance galvanometers, the fall in sensitivity due to heat becomes much larger, and extends to lower illuminations.

Cell *S*, on the other hand, behaved quite differently. At 59,000 metre candles a rise of 25° C. produced very little effect on the 10-ohm sensitivity, though for higher resistances the usual fall was apparent. At lower illuminations, however, the sensitivity decreased when hot, appreciably for 10 ohms and largely for 1010 ohms. This cell, accordingly, behaves like those tested by Teichmann (10), which showed a rise, both in the open circuit P.D. and in the short circuit current, when cooled below air temperature.

The results plotted in Fig. 3 show the importance of limiting the temperature rise as much as possible by the use of a water cell and by avoiding all unnecessary exposure of the cells to the sun.

Colour Sensitivity of the Cells.

Fig. 4 shows the colour sensitivities of the cells for low illuminations, as obtained with a simple form of spectrum projector which had previously been used for vacuum and gas-filled photo-electric cells and for ascertaining the

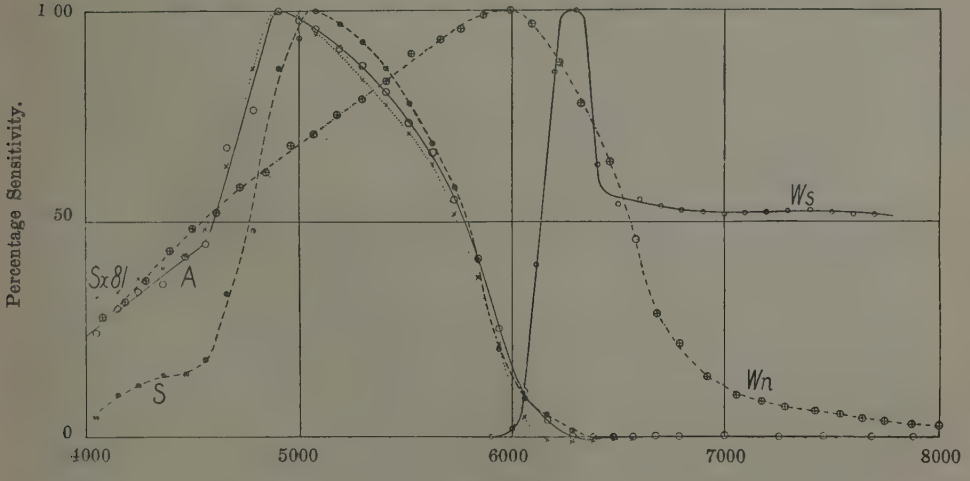


FIGURE 4.

transmission curves of various light filters (2). This gave a beam of cross-section about 2·4 cm. × 3·5 mm., whose total spectral range varied from about 50 Å at 4000 Å to about 250 Å at 7500 Å. The energy in this beam was measured with a Moll vacuum thermo-junction in the focus of a lens. The cell to be tested was mounted in such a way that it could quickly be interposed in the path of the beam in front of the thermo-junction lens, or withdrawn so as to allow the light to fall on the latter. Both cell and thermo-junction were connected to sensitive galvanometers, whose relative readings gave the sensitivity of the cell for weak light for the region of the spectrum in use. In plotting the readings for cell *Ws,* which shows a very sharp "cut off" of sensitivity in a region where the spectrum band used is rather wide, a correction has been introduced for the finite width of the band, so that the values shown correspond approximately to monochromatic light of the given wave-length. For the other cells this correction was negligible, and has not been applied, the average value for the entire band being plotted against the mean wave-length.

It will be seen that the curves for cells *A* and *Sx 81,* both of which are of the Auger type, are very similar, while that for *S,* also a cuprous oxide cell, does not differ very greatly, except that it shows no trace of a reverse effect in the red, which was noticeable in *A,* and, to a much greater degree, in *Sx 81.*

This reverse effect is irregularly distributed over the cell, being entirely absent from some small regions where small positive currents are obtained with light of wave-length about 6300 Å, which in other regions of the cell gives the maximum negative response, amounting to about 10 per cent. of the maximum positive sensitivity. The curve shown in Fig. 4 is for a strip about 3·5 mm. wide across the centre of the cell from rim to rim. This includes both positive and negative regions, so that the net negative effect shown in the figure is only about 1·5 per cent.

As the current flowing in the external circuit of these cuprous oxide cells in white light is from the copper back plate to the transparent film, it is probably a front surface effect of the cuprous oxide film. It is possible that sufficient red light may penetrate the thinner parts of the oxide to cause the back surface effect to preponderate, thus explaining both the reversal and its patchy nature.

This phenomenon was described, and the explanation set forth above was given, by Auger and Lapicque (4) in a paper which the authors kindly sent us. Unfortunately, when we came across the effect a year later we only remembered their curve, which does not show it, and so in a letter to *Nature* (9) did not refer to their work. For this oversight we can only offer apologies.

Alternatively, the effect may be due to the presence of cupric oxide, which Athanasiu (1) has observed to give a reverse E.M.F. in photo-voltaic cells. The rôle of the two oxides of copper in the rectifier cell has been discussed by M. Dubar (6).

It is evident from Fig. 4 that we can obtain rectifier cells suitable for observing any required part of the visible spectrum, while the curve for cell *Wn* is much closer to that of the eye than that for any type of cell which we have

hitherto come across. Its extension into the infra-red is only short and unimportant, which is an advantage for submarine measurements. These cells are said to have a considerable range of sensitivity in the ultra-violet when fitted with quartz windows.

The absolute value of the illumination to be deduced from the reading of any photo-electric cell will obviously depend on the colour sensitivity of the cell, and on the colour of the source used as a standard. We have hitherto adopted the vacuum potassium cell as a standard for the measurement of daylight. This, at least, appears to have the advantage of permanency over a period of years, but, as it is chiefly sensitive to blue light, and not at all to red, it would, if standardised against an incandescent lamp, give values for daylight illumination far in excess of visual estimates. We have, accordingly, hitherto used a carbon arc, the colour temperature of which is as high as 3780° K., as being the nearest approach to daylight that was easily reproducible with, at least, approximate accuracy. It also has the great advantage of readily supplying an intensity of illumination comparable with daylight; but as it is in many ways an unsatisfactory standard, we have considered adopting instead "Mean Noon Sunlight" obtained according to the specification of the Seventh International Congress of Photography (London, 1928).

The chief objection to this source, which consists of a filament lamp run at 2360° K. and two liquid colour filters, is that it is only possible to obtain relatively small intensities, so that it would not be suitable for use with any cell whose illumination current curve differed much from a straight line. Moreover, it is relatively weak in the violet and near ultra-violet, to which many cells are very sensitive.

Effect of Exposure and Time on the Sensitivities of the Cells.

Repeated tests on cells *A*, *Sx 81*, and *S* showed that the sensitivity of all three fell with lapse of time and exposure to bright sunlight. Thus, as a result of exposure on the laboratory roof for 10 days in bright June weather, the sensitivity of *A* fell by some 35 per cent. A further exposure for nearly two days caused little, if any, further drop; but, when tested again 8 months later, a further fall, ranging from 10 per cent. for weak light to 20 per cent. for strong light, had taken place, although the cell had not been much exposed to strong light in the meanwhile. Similarly, *Sx 81*, which had been used in the field, had lost 20 per cent. of its sensitivity in 8 months, the loss in this case being rather greater for weak light, and *S*, which had not been used out of doors, showed in the same period a drop which, like *A*, increased from about 10 per cent. for weak light to nearly 20 per cent. for strong light. We have not yet had an opportunity of carrying out similar tests on *Ws* to determine the constancy or otherwise of their behaviour.

The Bergmann (Weston) cell, *Wn*, on the other hand, has shown no appreciable change of sensitivity over a wide range of illumination after 33 days' continuous exposure to Spring daylight, including many hours of sunshine. Further tests are in progress.

SUMMARY.

The results may be summarised as follows:—

(1) In using rectifier cells for the measurement of daylight it is necessary to know the form of the current illumination curve for the particular cell, as this is rarely linear. This may be obtained by bench tests or by comparison in varying light of constant colour with a vacuum photo-electric cell.

(2) The form of the curve depends greatly on the resistance of the galvanometer used, and a low-resistance galvanometer must be used with most of the cells for the measurement of bright light. It is best to use a shunt designed so as to reduce the galvanometer sensitivity, when required, without affecting its resistance.

(3) Temperature effects are important with some cells, and should always be minimised by avoiding exposure of the cell to sunlight except for the brief period required for taking a reading. If longer exposures are required, a water cell may be interposed.

(4) It is possible to obtain cells suitable for measuring any part of the visible spectrum. Some cells of the Auger type show a small reverse effect in the red, which might in certain circumstances need to be allowed for in the interpretation of the results. The Bergmann cell is sensitive throughout the whole of the visible spectrum, its maximum sensitivity being in the orange red.

(5) No rectifier cell that we have tested over a period of several months has maintained its sensitivity unaltered. The Bergmann cell has, however, shown no change after 33 days' continuous exposure to daylight, and may possibly maintain its sensitivity indefinitely. As the change in sensitivity of a cell may vary with the intensity, it is desirable to restandardise at intervals for different illuminations. This may be done in daylight against a vacuum cell, but if so possible changes in the colour of the light must be remembered.

(6) Subject to the conditions and limitations given above, the cells would appear to be quite suitable for comparative measurements of daylight where approximate accuracy will suffice.

REFERENCES.

1. Athanasiu, G.—C.R. Acad. Sci., Paris, **195**, 767. 1932.
2. Atkins, W. R. G., and Poole, H. H.—Sci. Proc. R. Dub. Soc., **20**, 13. 1931.
3. Atkins, W. R. G., and Poole, H. H.—J. Marine Biol. Assoc., **18**. 1933. In the press.
4. Auger, P., and Lapicque, C.—C.R. Acad. Sci., Paris, **193**, 319. 1931.
5. Bergmann, L.—Phys. Zeits. **32**, 286. 1931.
6. Dubar, L.—C.R. Acad. Sci., Paris, **194**, 1322. 1932.
7. Lange, B.—Phys. Zeits. **31**, 139. 1930.
8. Poole, H. H., and Atkins, W. R. G.—J. Marine Biol. Assoc., **15**, 460. 1928.
9. Poole, H. H., and Atkins, W. R. G.—Nature, **131**, 133. Jan. 28, 1933.
10. Teichmann, H.—Zeits. f. Physik, **65**, 709. 1930.

SCIENTIFIC PROCEEDINGS.

VOLUME 20.

1.—A Modified Form of Radon Capillary Apparatus. By H. H. POOLE, Sc.D. (Plate 1.) 1s. (December, 1930.)

2.—The Exudation of Water from the Leaf-tips of *Colocasia antiquorum*, Schott. By HENRY H. DIXON, Sc.D., F.R.S., University Professor of Botany, Trinity College, Dublin, and G. JOLY DIXON. 6d. (December, 1930.)

3.—The Application of Gamma Radiation to Deep-seated Tumours.—II. By J. JOLY, Sc.D., F.R.S., F.T.C.D., Scientific Adviser to Dr. Steevens' Hospital. 6d. (December, 1930.)

4.—Photo-electric Measurements of Illumination in relation to Plant Distribution. Part 4. Changes in the Colour Composition of Daylight in the open and in shaded situations. By W. R. G. ATKINS, Sc.D., F.R.S., and H. H. POOLE, Sc.D. 3s. (April, 1931.)

5.—Observations on the Photo-electric Measurement of the Radiation from Mercury Vapour Lamps and from the Sun, and on the Effects of such Radiation upon the Skin. By W. R. G. ATKINS, Sc.D., F.R.S. 1s. 6d. (May, 1931.)

6.—Some Experiments on the Accuracy obtainable with Gas-filled Photo-electric Cells. By W. R. G. ATKINS, Sc.D., F.R.S. 6d. (May, 1931.)

7.—A Method of Distinguishing Certain Strains of New Zealand Perennial Ryegrass (*Lolium perenne*, L.) by Examination of Seedlings under Screened Ultra-violet Light. By P. A. LINEHAN, A.R.C.Sc.I., B.Agr.Sc., and S. P. MERCER, B.Sc. (AGR. LOND.), N.D.A., Ministry of Agriculture, The Queen's University, Belfast. (Plate 2.) 1s. (May, 1931.)

[Nos. 1 to 7, price 7s.]

8.—Award of the Boyle Medal to Sir John Purser Griffith, M.A.I., M.INST.C.E., *Past-President, Inst. C. E.*, 1931. (June, 1931.)

9.—A Study of Bacteria belonging to the Sub-Genus *Aerobacter*. By M. GRIMES and A. J. HENNERTY, Department of Dairy Bacteriology, University College, Cork. 6d. (July, 1931.)

10. Report of the Irish Radium Committee for the Year 1930. 1s. 6d. (September, 1931.)

11. On a Method of Distinguishing the Seedlings of Swedish Turnip (*Brassica napus* L. var. *Napobrassica* (L.) Reichb.) from those of Rape (*Brassica napus* L. var. *Biennis* (Schübl. et Mart.) Reichb.). By M. J. GORMAN, A.R.C.Sc.I., Department of Agricultural Botany, University College, Dublin, and H. A. LAFFERTY, F.R.C.Sc.I., Economic Botanist to the Department of Agriculture, Dublin. (Plate 3.) 1s. (September, 1931.)

12—The Influence of the Work of Sir William Rowan Hamilton on Modern Mathematical Thought. By PROFESSOR A. W. CONWAY, D.Sc., F.R.S. [Being a Paper read before the Special Bi-Centenary Scientific Meeting of the Royal Dublin Society, June 24th, 1931.] 6d. (September, 1931.)

[Nos. 8 to 12, price 3s.]

13.—On Alginic Acid: Its Mode of Occurrence and its Constitution. By THOMAS DILLON, M.A., D.Sc., and ANNIE MCGUINNESS, M.Sc., University College, Galway. 6d. (November, 1931.)

14.—The Performance of a Reservoir Subjected to Flood. By H. H. JEFFCOTT. 6d. (December, 1931.)

15.—Cytological Studies of Potato Plants affected with certain Virus Diseases. By PHYLLIS CLINCH, M.Sc., PH.D., Department of Plant Pathology, Albert College, Glasnevin, Dublin. [Being a Paper read before the Special Bi-Centenary Scientific Meeting of the Royal Dublin Society, June 24th, 1931.] (Plates 4 to 8.) 5s. (January, 1932.)

16.—The Effect of an Insufficient Supply of Vitamin D on the Growth of the Skeleton and Internal Organs of Chickens. By E. J. SHEEHY, F.R.C.Sc.I., B.Sc., Lecturer in Animal Nutrition, University College, Dublin, and MISS K. SHEIL, Superintendent of the Poultry Department. (Plate 9.) 1s. (January, 1932.)

17.—Report on the Recent Bog-Flow at Glencullin, Co. Mayo. By A. D. DELAP, A. FARRINGTON, R. LLOYD PRAEGER, and LOUIS B. SMYTH. 1s. (April, 1932.)

18.—A Critical Review of Some Recent Work on the Occurrence of Virus Complexes in the Potato. By PAUL A. MURPHY, Sc.D., Albert Agricultural College, Glasnevin, Dublin. 1s. 6d. (May, 1932.)

19.—Electrical Properties of Oil-Water Emulsions, with Special Reference to the Structure of the Plasmatic Membrane.—II. By HENRY H. DIXON, Sc.D., F.R.S., Professor of Botany in the University of Dublin, and T. A. BENNET-CLARK, M.A., PH.D., Lecturer in Botany in the University of Manchester. 1s. (April, 1932.)

20.—The Compound Nature of Crinkle, and its Production by Means of a Mixture of Viruses. By PAUL A. MURPHY, Sc.D., and ROBERT M'KAY, A.R.C.Sc.I., Albert Agricultural College, Glasnevin, Dublin. (Plates 10 to 12.) 2s. 6d. (May, 1932.)

[Nos. 13 to 20, price 12s.]

21.—Report of the Irish Radium Committee for the Year 1931. (Including Reports by Oliver Chance, M.B., B.CH., Richmond, Sir Patrick Dun's, and Coombe Hospitals; John A. Geraghty, M.B., Mater Misericordiae Hospital; Oswald J. Murphy, M.B., B.CH., B.A.O., St. Vincent's Hospital, Dublin; C. Conor O'Malley, B.SC., D.O.M.S., Central Hospital, Galway; Bethel Solomons, M.D., F.R.C.P.I., Master, Rotunda Hospital; Sir Robert Woods, M.B., M.CH., F.R.C.S.I.) 1s. 6d. (June, 1932.)

22.—The Inhibition of Chemical Reactions. Part 5. The Influence of Pyridine and other Substances on the Absorption of Ethylene by Sulphuric Acid, and on the Surface Tension of Sulphuric Acid. By WILLIAM SYDNEY EAGAR HICKSON, M.SC., and KENNETH CLAUDE BAILEY, SC.D., LITT.D., Fellow of Trinity College, Dublin. 1s. (June, 1932.)

23.—A Method for Automatically Recording the Oxygen Intake of Living Tissues. By T. A. BENNET-CLARK, M.A., PH.D., Lecturer in Botany in the University of Manchester. [Communicated by Professor H. H. Dixon, SC.D., F.R.S.] (August, 1932.)

24.—The Respiratory Quotients of Succulent Plants. By T. A. BENNET-CLARK, M.A., PH.D., Lecturer in Botany in the University of Manchester. [Communicated by Professor H. H. Dixon, SC.D., F.R.S.] (August, 1932.) [Nos. 23 and 24, 1s. 6d.]

25.—On the Cultivation in Artificial Media of *Catenaria anguillulae*, a Chytridiacean Parasite of the Ova of the Liver Fluke, *Fasciola hepatica*. By J. BAYLEY BUTLER, M.A., M.B., B.CH., B.A.O., Professor of Zoology, University College, Dublin, and ANNIE HUMPHRIES, M.SC., Assistant, Department of Zoology, University College, Dublin. (Plates 13–18.) 5s. (August, 1932.)

26.—Factors which Determine the Nutritive Value (Stock-carrying and Fattening Capacity) of Untreated Natural Pastures. By E. J. SHEEHY, F.R.C.SC.I., B.SC., Department of Animal Nutrition, University College, Dublin. 2s. (August, 1932.)

27.—A Comparison of Some European and American Virus Diseases of the Potato. By PAUL A. MURPHY, SC.D., and ROBERT MCKAY, A.R.C.SC.I., Albert Agricultural College, Glasnevin, Dublin. 1s. (August, 1932.)

[Nos. 21 to 27, price 10s.]

28.—The Anatomy of the Tortoise. By J. STUART THOMSON, M.SC., PH.D., F.R.S.E., formerly Senior Lecturer in Zoology in the Victoria University of Manchester. (Plates 19–43.) 8s. (December, 1932.)

29.—Effect of the Conditions of Storage on the Vitamin D Potency and on other Features of Codliver Oil. By E. J. SHEEHY, F.R.C.SC.I., B.SC., Lecturer in Animal Nutrition, University College, Dublin. 6d. (December, 1932.)

30.—A Suggested Mode of Radiotherapy when Long-continued Feeble Gamma Radiation may be Desirable. By J. JOLY, SC.D., F.R.S. 6d. (December, 1932.)

31.—On the Colloidal Calcium Phosphate of Milk. By G. T. PYNE and J. J. RYAN, Department of Dairy Chemistry, University College, Cork. [Communicated by Professor F. E. Hackett.] 6d. (December, 1932.)

32.—An Investigation of the Behaviour of Neon Discharge Tubes in a Flashing Capacity Circuit by Means of a Cathode Ray Oscillograph. By J. H. J. POOLE, SC.D. (Plate 44.) 1s. (January, 1933.)

33.—Bast-sap. By HENRY H. DIXON, SC.D., F.R.S., University Professor of Botany in Trinity College, Dublin. (Plate 45.) 1s. (January, 1933.)

34.—A Study of *Phoma Lingam* (Tode) Desm., and of the "Dry Rot" it Causes, Particularly in Swede Turnips. By WILLIAM HUGHES, B.AGR.SC., Albert Agricultural College, Glasnevin, Dublin. [Communicated by Dr. P. A. Murphy.] (Plates 46 and 47.) 3s. 6d. (February, 1933.)

35.—A Classification of the Biological Elements, with a Note on the Biochemistry of Beryllium. By WILLIAM ROBERT FEARON, M.B., SC.D., F.T.C.D., F.I.C. 6d. (February, 1933.)

36. Experiments on the Suitability of Some Rectifier Photo Cells for the Measurement of Daylight. By H. H. POOLE, SC.D., and W. R. G. ATKINS, SC.D., F.R.S., F.INST.P. 6d. (April, 1933.)

[Nos. 29 to 36, price 7s.]

PRINTED BY PONSONBY AND GIBBS, UNIVERSITY PRESS, DUBLIN.